CANCER
TREATMENT REPORTS

March 1986
Volume 70
Number 3
Pages 329-437

U.S.Department of Health
and Human Services
Public Health Service
National Institutes of Health

CANCER
TREATMENT REPORTS

National Cancer Institute, Vincent T. DeVita, Jr, ***Director***

International Cancer Information Center
Susan Molloy Hubbard, ***Director***

Publications Branch
Jean Griffin Baum, ***Chief***

Robert E. Wittes, ***Editor-in-Chief***
Dee Kittner LeRoy, ***Managing Editor***

Editorial Board

Joseph Aisner
Lewis Aronow
Gregory Curt
Richard L. Cysyk
Ross C. Donehower
Michael Friedman
Timothy J. Kinsella
Robert F. Ozols
Carl Pinsky
David G. Poplack
Richard Simon

Advisory Board

Charles M. Balch
Robert C. Bast, Jr
Gianni Bonadonna
Edwin C. Cadman
Giulio J. D'Angio
I. David Goldman
Heine H. Hansen
Simon Kramer
Susan E. Krown
John E. Niederhuber
T. L. Phillips
H. M. Pinedo
Carol S. Portlock
Joseph V. Simone
Samuel A. Wells, Jr

Editorial Staff

Editors:
Elaine Price Beck
Maralyn Farber Berlin
Mary Olvey Cowgill

Editorial Assistant:
Julia Redmond

Manuscript Office:
Sally Bernstein
Kathleen Sager
Olga Sisson

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports*. Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 231, National Cancer Institute, Bethesda, MD 20892 (301-496-6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors," which appears on the second page of every issue.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402.

INSTRUCTIONS TO AUTHORS

Title Page
Includes complete title of the manuscript and the full name, affiliation, and address of each author. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary
Begin the article with a short summary.

Text
Submit an original and 3 copies of the manuscript, typed double-spaced. Number each page consecutively. Dot-matrix or italicized texts will not be accepted. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use only standard abbreviations; otherwise use full words.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus*: author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, chapter title, book title, city and name of publisher, date of publication, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers published in meeting proceedings should include author; title of paper; title of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs should be clear, glossy prints. If original art work is submitted, it should be done in black ink for good reproduction. Please send 2 copies of each print. Photocopies reproduce poorly. Color photographs or transparencies are acceptable.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Brief Reports
Manuscripts published in the Brief Reports section should be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor should be no longer than 3 double-spaced text pages and should be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Summaries
Manuscripts published in this section should be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints
Supplied free of charge, 100 reprints (20 reprints for Letters to the Editor) will be sent to the senior or specified author about 3 weeks after publication of the article.

Announcements
Announcements of meetings are accepted for publication free of charge. Mail to: Editorial Office, Cancer Treatment Reports, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 231, National Institutes of Health, Bethesda, MD 20892.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1987.

METHODOLOGIC GUIDELINES FOR REPORTS OF CLINICAL TRIALS*

1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

2. All patients registered on study should be accounted for. The report should specify for each treatment the number of patients who were not eligible, who died or withdrew before treatment began. The distribution of follow-up times should be described for each treatment, and the number of patients lost to follow-up should be given.

3. The study should not have an inevaluability rate for major endpoints of greater than 15%. Not more than 15% of eligible patients should be lost to follow-up or considered inevaluable for response due to early death, protocol violation, missing information, etc.

4. In randomized studies, the report should include a comparison of survival and/or other major endpoints for all eligible patients as randomized, that is, with no exclusions other than those not meeting eligibility criteria.

5. The sample size should be sufficient to either establish or conclusively rule out the existence of effects of clinically meaningful magnitude. For "negative" results in therapeutic comparisons, the adequacy of sample size should be demonstrated by either presenting confidence limits for true treatment differences or calculating statistical power for detecting differences. For uncontrolled phase II studies, a procedure should be in place to prevent the accrual of an inappropriately large number of patients, when the study has shown the agent to be inactive.

6. Authors should state whether there was an initial target sample size and, if so, what it was. They should specify how frequently interim analyses were performed and how the decisions to stop accrual and report results were arrived at.

7. All claims of therapeutic efficacy should be based upon explicit comparison with a specific control group, except in special circumstances where each patient is his own control. If nonrandomized controls are used, the characteristics of the patients should be presented in detail and compared to those of the experimental group. Potential sources of bias should be adequately discussed. Comparison of survival between responders and nonresponders does not establish efficacy and should not generally be included. Reports of phase II trials which draw conclusions about antitumor activity but not therapeutic efficacy generally do not require a control group.

8. The patients studied should be adequately described. Applicability of conclusions to other patients should be carefully dealt with. Claims of subset-specific treatment differences must be carefully documented statistically as more than the random results of multiple-subset analyses.

9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

---

* *From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

Contents

Cancer Treatment Reports
Volume 70, Number 3
March 1986

Clinical Studies

329 Treatment of Metastatic Malignant Melanoma With Vinblastine, Dacarbazine, and Cisplatin: A Report From the Cancer and Leukemia Group B. **R. W. Carey, J. R. Anderson, M. Green, R. R. Ellison, L. Nathanson, and B. J. Kennedy.** Cancer and Leukemia Group B

333 Cisplatin and Vinblastine Chemotherapy for Metastatic Non-Small Cell Carcinoma Followed by Irradiation in Patients With Regional Disease. **R. H. Blum, J. Cooper, A. M. Schmidt, R. Ashinoff, A. Collins, J. C. Wernz, J. L. Speyer, A. Boyd, and F. M. Muggia.** New York University Medical Center, School of Medicine, New York, New York

339 Combination Chemotherapy With Vindesine, Etoposide, and Cisplatin in Non-Small Cell Lung Cancer: A Pilot Study of the Southeastern Cancer Study Group. **J. D. Hainsworth, L. L. Porter, III, D. H. Johnson, K. R. Hande, S. N. Wolff, R. Birch, G. Enas, and F. A. Greco.** Southeastern Cancer Study Group

343 Salvage Chemotherapy in Hodgkin's Disease Irradiation Failures: Superiority of Doxorubicin-Containing Regimens Over MOPP. **A. Santoro, S. Viviani, C. J. R. Villarreal, V. Bonfante, A. Delfino, P. Valagussa, and G. Bonadonna.** Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

Phase I–II Trials

349 Electrocardiographic Monitoring of Patients Receiving Phase I Cancer Chemotherapy. **M. R. Hersh, W. Linn, J. G. Kuhn, and D. D. Von Hoff.** University of Texas Health Science Center at San Antonio and the Audie L. Murphy Memorial Veterans Hospital, San Antonio

353 Phase I–II Evaluation of Intra-arterial Diaziquone for Recurrent Malignant Astrocytomas. **H. S. Greenberg, W. D. Ensminger, P. B. Layton, S. Gebarski, M. Meyer, B. Chaffee, J. F. Bender, and A. J. Grillo-Lopez.** The University of Michigan Hospitals, Ann Arbor

359 Disposition of Tricyclic Nucleoside-5′-monophosphate in Blood and Plasma of Patients During Phase I and II Clinical Trials. **G. Powis, P. J. Basseches, D. M. Kroschel, R. L. Richardson, M. J. O'Connell, and L. K. Kvols.** Mayo Clinic and Foundation, Rochester, Minnesota

363 Phase I–II Study of Continuous-Infusion High-Dose Human Lymphoblastoid Interferon and the In Vitro Sensitivity of Leukemic Progenitors in Nonlymphocytic Leukemia. **J. Mirro, L. W. Dow, D. K. Kalwinsky, G. V. Dahl, P. Weck, J. Whisnant, and S. B. Murphy.** St. Jude Children's Research Hospital, Memphis, Tennessee

369 Ethinyl Estradiol and Medroxyprogesterone Acetate in Patients With Epithelial Ovarian Carcinoma: A Phase II Study. **R. S. Freedman, P. B. Saul, C. L. Edwards, C. J. Jolles, D. M. Gershenson, L. A. Jones, E. N. Atkinson, and W. J. Dana.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

375 Phase II Studies of Homoharringtonine in Patients With Advanced Malignant Melanoma; Sarcoma; and Head and Neck, Breast, and Colorectal Carcinomas. **J. A. Ajani, I. Dimery, S. P. Chawla, K. Pinnamaneni, R. S. Benjamin, S. S. Legha, and I. H. Krakoff.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Pharmacology

381 Effect of Tamoxifen on Immune Functions. **H. Joensuu, A. Toivanen, and E. Nordman.** University Central Hospital of Turku, Finland

383 Pentamethylmelamine: Review of an Aqueous Analog of Hexamethylmelamine. **B. J. Foster, K. Clagett-Carr, D. Hoth, and B. Leyland-Jones.** National Cancer Institute, Bethesda, Maryland

391 Metalloporphyrin Contrast Agents for Magnetic Resonance Imaging of Human Tumors in Mice. **N. J. Patronas, J. S. Cohen, R. H. Knop, A. J. Dwyer, D. Colcher, J. Lundy, F. Mornex, P. Hambright, M. Sohn, and C. E. Myers.** Clinical Center, National Institutes of Health, Bethesda, Maryland

397 Growth Inhibition and Eicosanoid Metabolism in the R3230AC Mammary Adenocarcinoma by Interferon. **R. A. Karmali, H. Schellekens, F. Lefevre, J. Marsh, C. Fuchs, and M. Krim.** Rutgers, The State University of New Jersey, New Brunswick

401 Minimal Surgical Debridement for the Treatment of Chemotherapeutic Agent-Induced Skin Extravasations. **T. S. Loth.** Irwin Army Community Hospital, Fort Riley, Kansas

BRIEF REPORTS

405 Phase II Evaluation of Doxorubicin Plus Cisplatin in Advanced Thyroid Cancer: A Southeastern Cancer Study Group Trial. **S. D. Williams, R. Birch, and L. H. Einhorn.** Southeastern Cancer Study Group

409 Lack of Synergy Between Cisplatin and Cytarabine Against Ovarian Carcinoma In Vitro. **S. B. Howell and S. Gill.** University of California, San Diego, La Jolla

411 Effect of 1-Methyl-5-sulfonamide-4-nitroimidazole and 1-Methyl-5-bromide-4-nitroimidazole on the Radiosensitivity of EMT6 Tumor Cells. **S. Rockwell, C. G. Irvin, and M. Nierenburg.** Yale School of Medicine, New Haven, Connecticut

CLINICAL TRIALS SUMMARIES

415 Cisplatin in Patients With Gastric Cancer: A Cancer and Leukemia Group B Phase II Study. **M. C. Perry, M. R. Green, R. Mick, and P. Schein.** Cancer and Leukemia Group B

417 Phase II Trial of Aminothiadiazole in Advanced Non-Small Cell Lung Cancer. **D. H. Shevrin, W. P. McGuire, V. Lanzotti, S. Krauss, R. Blough, M. Cobleigh, C. Johnson, T. Lad, and L. Kukla.** University of Illinois at Chicago

419 Phase II Study of Sequential Methotrexate and 5-FU Combination in the Treatment of Advanced Colorectal Cancer. **L. Canobbio, M. T. Nobile, A. Ardizzoni, R. Tatarek, and R. Rosso.** Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

421 Phase II Trial of Carboplatin in Non-Small Cell Lung Cancer. **I. N. Olver, R. C. Donehower, D. A. Van Echo, D. S. Ettinger, and J. Aisner.** University of Maryland Cancer Center, Baltimore

423 Phase II Trial of Bisantrene in Previously Treated Patients With Ovarian Carcinoma: A Southwest Oncology Group Study. **J. D. Cowan, E. A. Surwit, D. S. Alberts, J. G. Boutselis, and B. A. Neilan.** Southwest Oncology Group

425 Phase II Trial of Fludarabine in Patients With Epithelial Ovarian Cancer. **J. J. Kavanagh, C. A. Stringer, L. J. Copeland, D. M. Gershenson, and P. Saul.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

427 Phase II Trial of Diaziquone in Anthracycline-Resistant Adult Soft Tissue and Bone Sarcoma Patients: A Southeastern Cancer Study Group Trial. **C. Chan, A. Bartolucci, D. Brenner, C. Presant, E. Davila, J. Carpenter, F. A. Greco, G. Clamon, and J. Moore.** Southeastern Cancer Study Group

429 Phase II Trial of Diaziquone in Malignant Mesothelioma. **R. T. Eagan, S. Frytak, R. L. Richardson, E. T. Creagan, and W. C. Nichols.** Mayo Clinic, Rochester, Minnesota

431 High-Dose Medroxyprogesterone Acetate in Patients With Renal Adenocarcinoma and Measurable Lung Metastases: A Phase II Study. **M. Kjaer and P. L. Frederiksen.** Finsen Institute, Copenhagen, Denmark

LETTERS

433 Cardiotoxicity Associated With 4′-Deoxydoxorubicin. **D. Blayney.** City of Hope National Medical Center, Duarte, California

433 Response of Glucagonoma Syndrome to Lomustine. **J. D. Khandekar and P. Sriratana.** Evanston Hospital, Illinois, and Northwestern University Medical School, Chicago, Illinois

435 ANNOUNCEMENTS

Treatment of Metastatic Malignant Melanoma With Vinblastine, Dacarbazine, and Cisplatin: A Report From the Cancer and Leukemia Group B[1,2]

Robert W. Carey,[3,*] James R. Anderson,[4] Mark Green,[5] Rose Ruth Ellison,[6] Larry Nathanson,[7] and B. J. Kennedy[8]

Chemotherapy for malignant melanoma with the combination of vinblastine (VBL), bleomycin, and cisplatin (DDP) (VBD) has been reported to produce objective regression (complete or partial remissions) in 43% (1), 11% (2), 0% (3), 19%–22% (4), and 10% (5) of patients. The reasons for such variability in response rates are obscure; York et al (3) have emphasized the notorious unpredictability of this disease. The VBD combination produces considerable toxicity. Nathanson et al (1) found an 86% incidence of acute gastrointestinal toxicity (severe in 20% of patients, including duodenal ulcer), a 67% incidence of delayed gastrointestinal toxicity, primarily ileus obstipation (severe in 15%), and a 52% incidence of mucositis (severe in 10%). Investigators at the National Cancer Institute of Canada have concluded that the short duration of response and the severe toxicity make VBD unsuitable first-line treatment for patients with metastatic melanoma (4). In an effort to find a chemotherapy program with better patient tolerance and efficacy comparable or superior to VBD, the Cancer and Leukemia Group B (CALGB) carried out a pilot study substituting dacarbazine (DTIC) for bleomycin (VDD).

MATERIALS AND METHODS

The CALGB pilot study of VDD was opened to patient entry in September 1981. To be eligible for entry in the study, patients were required to have histologic proof of melanoma with advanced local, regional, or metastatic disease. Only patients with no prior chemotherapy (excluding perfusion) and performance status of 0–2 (< 50% time in bed) were eligible. Two weeks must have elapsed since any prior radiotherapy except for treatment of the central nervous system. Patients must have had measurable disease, wbc count > 4000/μl, platelet count > 100,000/μl, creatinine < 2 mg/dl, BUN < 30 mg/dl, bilirubin < 2 mg/dl, and SGOT/SGPT less than 6 times normal.

Patients were not eligible for entry in the study if the recurrent disease was potentially amenable to surgical care, if they were candidates for perfusion therapy, or if central nervous system or bone was the only measurable disease site. Patients with a history of prior malignancy other than basal cell cancer of the skin or cancer in situ of the cervix were also ineligible.

The VDD schedule consisted of 5 mg/m^2 of VBL by iv push on Days 1 and 2, 150 mg/m^2 of DTIC on Days 1–3 as a 15–30-minute bolus in 100 ml of 5% dextrose in water (D5W), and 50 mg/m^2 of DDP iv on Day 3. The DDP was preceded by hydration with D5W in one-half normal saline for 12–18 hours, with additional hydration, 12.5 mg of mannitol by iv push, and appropriate antiemetics. Cycles were repeated every 28 days and doses were decreased depending on the degree of hematologic toxicity experienced.

Responses were defined as follows: *complete response (CR)*—the complete disappearance of all clinical evidence of active tumor, lasting at least 4 weeks; *partial response (PR)*—a ⩾ 50% decrease in the sum of the products of measured lesions, lasting at least 4 weeks; *disease im-*

[1]Received Jan 22, 1985; revised June 3, 1985; accepted June 6, 1985.

[2]This study was conducted by the Cancer and Leukemia Group B (*Emil Frei, III*, chairman, CA-31946) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in this study: Wilmington Medical Center, DE (*I. Berkowitz*); Bowman Gray School of Medicine, Winston-Salem, NC (*M. R. Cooper*, CA-03927); McGill Cancer Center, Montreal, Quebec, Canada (*J. L. Hutchinson*, CA-31809); Mt. Sinai School of Medicine, New York, NY (*J. F. Holland*, CA-04475); Rhode Island Hospital, Providence (*L. Leone*, CA-08025); State University of New York Affiliated Hospitals, New York (*S. Rafla*); Walter Reed Army Medical Center, Washington, DC (*R. Weiss*, CA-26806); West Virginia University Medical Center, Morgantown (*P. Raich*); Long Island Jewish Hillside Medical Center, New Hyde Park, NY (*K. Rai*, CA-11028); Massachusetts General Hospital, Boston (*R. W. Carey*, CA-12449); Columbia University, New York, NY (*R. R. Ellison*, CA-12011); University of California, San Diego (*M. Green*, CA-11789); University of Missouri, Columbia (*M. Perry*, CA-12046); University of Minnesota, Minneapolis (*B. Peterson*, CA-16450); University of Maryland Cancer Center, Baltimore (*J. Aisner*, CA-31983); Virginia Mason Medical Clinic, Seattle, WA (*A. Einstein, Jr*); and Ochsner Clinic, New Orleans, LA (*C. Kardinal*).

[3]Massachusetts General Hospital, Boston.

[4]Department of Biostatistics, Harvard School of Public Health, Boston, MA.

[5]University Hospital, University of California, San Diego.

[6]Columbia-Presbyterian Medical Center, New York, NY.

[7]Nassau Hospital, Mineola, NY.

[8]University of Minnesota Hospitals, Minneapolis.

[*]*Reprint requests to:* R. W. Carey, MD, Medical Oncology Unit, Massachusetts General Hospital, The Cox Bldg, 100 Blossom St, Boston, MA 02114.

provement—tumor regression of a < 50% decrease in the sum of the products of measured lesions; *stable disease*—a < 25% decrease in the sum of the products of measured lesions; and *progressive disease*—a > 25% increase in the sum of the products of measured lesions.

RESULTS

Sixty-eight patients were entered in the study prior to closure in December 1982. Four patients have been excluded from this analysis (one never started treatment, one had no follow-up records, and two were ineligible [one with a performance status of 3 and one with a prior history of bladder cancer]). The age distribution of the 64 eligible patients was: < 40 years, 22%; 40–49 years, 20%; 50–59 years, 25%; and > 60 years, 33%. Fifty-nine percent of the patients were male, and 87% of the patients were ambulatory (performance status 0 or 1).

Five patients were unevaluable for response. Four patients received one course of therapy or less and were then lost to further follow-up. One patient refused chest x-ray necessary to evaluate response. Of the remaining 59 evaluable patients, 54 received full doses of VBL, 50 received full doses of DTIC, and 46 received full doses of DDP. There was excellent compliance with drug scheduling.

Drug Toxicity

Toxic effects experienced during treatment were mainly hematologic. Fifteen patients (23%) developed severe leukopenia (wbc count, 1000–1999/mm^3); in three (5%) it was considered life-threatening (wbc count, 1000–1999/mm^3). There were no treatment-related deaths. Ten patients (16%) had severe nausea and vomiting.

Response to Therapy

The best response to therapy among the 59 patients evaluable for response was as follows: CR, three patients (5%); PR, 12 (20%); disease improvement, seven (12%); stable disease, two (3%); and progressive disease, 35 (59%).

Sites of tumor response in the 15 patients who achieved CR or PR included: liver, two; bone, two; subcutaneous, three; lung, ten; lymph nodes, eight; and brain, none.

The durations of the three CRs measured from start of response were 289+, 299, and 355+ days; these responses were subcutaneous, lung, and lymph nodes; subcutaneous and bone; and subcutaneous and lung, respectively. Durations of PR varied from 63 to 371+ days.

An additional CR of lung metastases occurred in the patient excluded from analysis as ineligible because of prior bladder cancer.

For the 64 eligible patients, survival from entry in the study was estimated to be 58% at 6 months and 27% at 1 year.

DISCUSSION

The overall response rate of 25% (CR + PR) for VDD is somewhat higher than that recently reported for VBD (2–5), with considerable improvement in patient tolerance and compliance and considerably less mucocutaneous and pulmonary toxicity. The VBD combination had been reported to have a 43% objective response rate in metastatic melanoma (1), but others have not confirmed these results. Creagan et al (2) reported two responses among 18 patients (11%); York et al (3) found no responses among 20 evaluable patients; the National Cancer Institute of Canada (4) reported 12 responses among 64 patients (19%); and Luikart et al (5) reported four responses among 41 patients (10%). Most of these patients had received no prior chemotherapy. Mechl et al (6) reported seven responses among 17 evaluable patients (41%) with VBD using a higher dose of DDP, but the mean duration of therapeutic response was only 4 months. It is unusual to observe such a large number of visceral lesion responses. Comis (7) reports four of 21 patients receiving lomustine showing regression of pulmonary lesions and mentions that a three-drug program (DTIC, BCNU, and vincristine) showed a higher proportion of visceral response (17 of 20 patients) than DTIC alone (ten of 19). The VDD program in the current study is comparable in that 14 of 25 responses were of visceral disease, including two in the liver and two in the bone, sites which rarely respond to treatment.

The response rate obtained with VDD here is also somewhat higher than that which can be obtained utilizing DTIC alone in full therapeutic dose. In a randomized prospective trial in patients with metastatic malignant melanoma, about one-half of whom had received no prior chemotherapy, Luikart et al (5) found similar objective response rates for DTIC alone (14%) and VBD (10%). However, previously reported objective response rates to DTIC alone range between 16% and 31% in larger series (7). A randomized trial of VDD versus DTIC would be of interest, given the variability in patient characteristics, the evidence seen in this study for substantial regression of visceral disease, and the marked improvement in toxicity of VDD over VBD.

In conclusion, VDD is a well-tolerated chemotherapy regimen producing objective regression in previously untreated patients with metastatic unresectable malignant melanoma at a frequency somewhat greater than that customarily reported for DTIC alone. The VDD regimen appears suitable for induction therapy of patients with visceral metastatic melanoma, but a prospective randomized trial will be required to determine if it is clearly superior to DTIC alone. The CALGB may consider such a trial in the future.

REFERENCES

1. NATHANSON L, KAUFMAN SD, and CAREY RW. Vinblastine infusion, bleomycin, and *cis*-dichlorodiammine-platinum chemotherapy in metastatic melanoma. Cancer 48:1290-1294, 1981.
2. CREAGAN ET, AHMANN DL, SCHUTT AJ, ET AL. Phase II study of the combination of vinblastine, bleomycin, and cisplatin in advanced malignant melanoma. Cancer Treat Rep 66:567-569, 1981.
3. YORK RM, LAWSON DH, and MCKAY J. Treatment of metastatic malignant melanoma with vinblastine, bleomycin by infusion and cisplatin. Cancer 52:2220-2222, 1983.
4. CANADIAN MELANOMA GROUP. Vinblastine, bleomycin, and cisplatinum for the treatment of metastatic malignant melanoma. J Clin Oncol 2:131-134, 1984.
5. LUIKART SD, KENNEALEY GT, and KIRKWOOD JM. Randomized phase III trial of vinblastine, bleomycin, and *cis*-dichlorodiammine-platinum versus dacarbazine in malignant melanoma. J Clin Oncol 2:164-168, 1984.
6. MECHL Z, NEKULOVA M, SOPKOVA B, ET AL. The VBD regimen (vinblastine, bleomycin, *cis*-platinum) with high doses of *cis*-platinum in the therapy of advanced malignant melanoma. *In* Proceedings of the 13th International Congress of Chemotherapy, Vienna, 1983, pp 22-25.
7. COMIS RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165-176, 1976.

Cisplatin and Vinblastine Chemotherapy for Metastatic Non-Small Cell Carcinoma Followed by Irradiation in Patients With Regional Disease[1,2]

Ronald H. Blum,* Jay Cooper, Ann Marie Schmidt, Robin Ashinoff, Amy Collins, James C. Wernz, James L. Speyer, Arthur Boyd, and Franco M. Muggia[3]

Forty-four patients with non-small cell carcinoma of the lung were treated every 3 weeks with vinblastine (4 mg/m^2/day iv × 2) and cisplatin (20 mg/m^2/day iv × 3). Of the 28 patients with metastatic disease, eight (29%; 90% confidence interval of true response, 17%–47%) achieved objective response, for a median duration of 27 weeks. Median survival in this group was 47 and 28 weeks for responders and nonresponders, respectively. Of the 16 patients with advanced regional disease, 11 (69%; 90% confidence interval of true response, 49%–86%) achieved objective response. Thirteen of these patients received consolidation radiotherapy (4500 cGy/25 fractions/5 weeks), with a boost of 1000 cGy/5 fractions/1 week in those patients who achieved response. In the three patients who did not receive radiotherapy, two died during the induction phase, one from grade 4 leukopenia and sepsis and the second from unrelated factors. The third patient had systemic progression of disease during induction chemotherapy. Six patients experienced overall improvement in their chemotherapy response from the radiotherapy. Two patients who did not respond to the chemotherapy achieved partial response with irradiation. Four patients who had partial response to the chemotherapy achieved complete response with irradiation, and seven patients had no further change in their degree of response to irradiation. The overall median survival of this group was 81 weeks. Maintenance chemotherapy was not given. After radiotherapy, the site of first failure was outside the radiation field in nine of 13 patients (69%). Hematologic toxicity was dose-limiting. Other toxic effects that were not dose-limiting included nephrotoxicity, neurotoxicity, and acute nausea and vomiting. In the patients with advanced regional disease, there was no increase in the radiation toxicity attributable to the chemotherapy. We conclude that: (*a*) this dose schedule of vinblastine and cisplatin has reproducible activity in non-small cell carcinoma of the lung; (*b*) the response and median survival of patients with advanced regional disease are superior to those of patients with metastatic disease; and (*c*) in patients with advanced regional disease, treatment with chemotherapy followed by radiotherapy yielded an overall response rate of 81% (90% confidence interval of true response, 60%–93%) and improved survival compared to a similar group of patients studied by others receiving radiotherapy alone. We recommend further testing of this concept. [Cancer Treat Rep 70:333–337, 1986]

Historically, chemotherapy for non-small cell carcinoma of the lung has been minimally effective. However, Gralla et al (1) reported encouraging results with the combination of vindesine and cisplatin. In trials as single agents, response rates of 22%–29% (2–6) and 9%–26% (7–11), respectively, have been reported. The 43% response rate of both drugs given concurrently suggested enhancement. Since vindesine, an investigational new drug, was not readily available, we initiated this clinical trial with the related vinca alkaloids vinblastine and cisplatin in June 1981 (12). We also sought to develop a treatment suitable for an ambulatory care setting.

Another concept tested by this trial was the use of chemotherapy for patients with advanced regional disease (13). Rather than receiving initial standard pallia-

[1]Received Feb 28, 1985; revised June 3, 1985; accepted June 13, 1985.

[2]Supported in part by Public Health Service grant CA-16087 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Divisions of Medical Oncology, Radiation, and Surgery, Rita and Stanley H. Kaplan Cancer Center, New York University Medical Center, School of Medicine, New York, NY.

**Reprint requests to:* Ronald H. Blum, MD, New York University Medical Center, School of Medicine, 550 First Ave, New York, NY 10016.

tive radiation therapy, patients who had advanced regional disease received vinblastine and cisplatin chemotherapy induction followed by consolidation with radiation therapy. This approach was based on the following concepts: (*a*) the high systemic failure rate for patients treated with regional radiation (14); (*b*) the relatively poor tolerance of chemotherapy given after radiation therapy; and (*c*) achieving higher response rates by using the tactic of "neoadjuvant chemotherapy," described by Frei (15). We report the initial response rate to chemotherapy for all patients and, for patients with advanced regional disease, the further response to radiation.

PATIENTS AND METHODS

All patients were treated at the New York University Medical Center from June 1981 through September 1984. Patient selection criteria included pathological proof of unresectable non-small cell carcinoma of the lung, no prior chemotherapy, Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, and normal marrow reserve and renal and hepatic functions. All patients were required to have measurable or evaluable disease (16). Response criteria are those defined by Eagan et al (16), and ECOG criteria are used in reporting treatment-related toxicity (17). Patients who had major regional morbidity, such as hemoptysis and postobstructive pneumonia, were excluded since they were immediately irradiated.

Staging criteria are those of the Radiation Therapy Oncology Group (RTOG) modification of the American Joint Committee (18). Staging procedures to distinguish advanced regional versus metastatic disease included pretherapy history, physical examination, chest roentgenogram, computerized axial tomography of the chest, bronchoscopy with or without mediastinoscopy, cbc, 12-channel biochemical profile, serum electrolytes, serum creatinine, radionuclide bone and liver scans, and computerized axial tomography of the brain or radionuclide brain scan.

Chemotherapy was administered to all patients as follows. Vinblastine was given as a rapid iv infusion at a dose of 4 mg/m^2/day for the first 2 days. Cisplatin at a dose of 20 mg/m^2/day iv in 1000 ml of 5% dextrose and 0.33% normal saline was given over 2 hours for 3 days. Pre- or post-infusion hydration was not given unless vomiting was severe. Antiemetic treatment was individualized for each patient, but generally included prochlorperazine, lorazepam, and dexamethasone. Cycles were repeated every 3 weeks. The duration of treatment in patients with metastatic disease, ie, T_1-T_4, N_0-N_3, and M_1-M_3, was to objective progression or limiting toxicity.

Sixteen patients with advanced regional disease would receive four courses of chemotherapy or until there was apparent maximal response, progression of disease, or limiting toxicity. These patients were M_0 with T_3N_2 (nine patients), T_2N_2 (four), T_1N_2 (one), and T_3N_1 (one), and M_1, exclusively on the basis of involved ipsilateral supraclavicular lymph nodes, ie, $T_3N_3M_1$ (one). Radiation therapy was planned to deliver 4500 cGy/25 fractions/5 weeks to a field encompassing the initial primary tumor and mediastinal-hilar lymph nodes, with a margin of 2 cm. For those patients achieving response, a boost of 1000 cGy/5 fractions/1 week to the prechemotherapy-sized primary lung tumor and grossly involved nodal regions was given when possible. All patients were simulated, and computerized axial tomography of the chest was used in their treatment planning.

RESULTS

Forty-four patients, 28 with metastatic disease and 16 with advanced regional disease, met the eligibility criteria and were evaluable for response. The median age was 59 years (range, 41-78), with 27 men and 17 women in the trial. One patient had a performance status of 0, 35 had a performance status of 1, and eight had a performance status of 2. The histologic types of non-small cell lung cancer were: 29 patients with adenocarcinoma, 11 with squamous cell carcinoma, two with large cell carcinoma, and two were classified as other or mixed. Six of the patients with metastatic disease had received prior irradiation and five had had prior curative-intent surgery. None of the patients with advanced regional disease had received prior treatment.

For patients with metastatic disease, the median number of courses of chemotherapy was four (range, one to ten). Responses and responder characteristics are shown in table 1. Of the 17 patients with measurable disease, one (6%) achieved complete response and four (24%)

TABLE 1.—Characteristics of responders with metastatic disease

Disease	No. of patients (%)	Cell type	Prior treatment
Measurable (17 patients)			
Complete response	1 (6)	Adenocarcinoma	Surgery and radiation
Partial response	4 (24)	3 adenocarcinoma; 1 squamous cell carcinoma	Surgery and radiation in 1 patient with adenocarcinoma
Evaluable (11 patients)			
Regression	3 (27)	2 adenocarcinoma; 1 squamous cell carcinoma	No prior treatment in any patient

achieved partial response. Of the 11 patients with evaluable disease, three (27%) achieved an evaluable regression. The overall response rate was 29%, with a median duration of 27 weeks. The median survival of the entire population was 28 weeks, but 47 and 26 weeks for responders and nonresponders, respectively.

Sixteen patients with advanced regional disease received a median of four courses of induction chemotherapy (range, three to six). Patient responses and characteristics are shown in table 2. Of the eight patients with measurable disease, one (13%) achieved complete response and three (38%) achieved partial response. Of the eight patients with evaluable disease, one (13%) achieved complete response and six (75%) achieved evaluable regression. The overall response rate to the induction chemotherapy was 69%.

Thirteen of the 16 patients with advanced regional disease received irradiation. Of the three patients who did not receive irradiation, two died during the induction phase, one from grade 4 leukopenia and sepsis and the other from unrelated factors, and the third from systemic progression of disease during induction chemotherapy. For the other patients, the dose of radiation delivered was 5500 cGy in five patients, with the final 1000 cGy delivered to gross disease only, 4500 cGy in three patients, with no cone down possible, and < 4500 cGy in five patients, because the extent or position of the tumor dictated only palliative radiation.

Six patients had improvement in their chemotherapy response by the irradiation. Two patients who did not respond to the chemotherapy achieved partial response with irradiation. Four patients who had partial response to chemotherapy achieved complete response with irradiation, and seven patients who had responded to chemotherapy had no further change in their degree of response with irradiation. The median duration of response and median survival grouped by best response were 81 and 112 weeks, respectively, for the five complete responders, and 29 and 50 weeks, respectively, for the eight partial responders. The overall median survival for the advanced regional disease group was 81 weeks.

After irradiation, the site of first failure was outside the radiation field in nine of 13 patients (69%): four in the brain, three in the lung, and two in the bone. Two patients had infield relapse despite radiation therapy, and one patient had simultaneous infield and outfield relapse. One patient died from respiratory failure without documented recurrence.

Toxicity

Toxicity is reported according to the worst grade per patient as a percent of the number of patients evaluable. Hematologic toxicity was dose-limiting. Leukopenia was grade 1 in 14% of the patients; grade 2 in 25%; grade 3 in 25%; and grade 4 in 11%. Drug-induced anemia was grade 1 in 25% of the patients; grade 2 in 18%; grade 3 in 0%; and grade 4 in 10%. Thrombocytopenia was grade 1 in 3% of the patients; grade 2 in 6%; and grade 3 in 8%. Clinically significant neurotoxicity was detected in 4% of the patients. Grade 2 nephrotoxicity was noted in 15% of the patients and grade 4 was noted in 5%. Acute nausea and vomiting was readily controlled with conventional antiemetics. Gastrointestinal toxicity was not dose-limiting in any patient.

In patients with advanced regional disease, the toxicity of chemotherapy versus radiation therapy to the chest was difficult to evaluate. Overall, there did not seem to be any increase in the radiation toxicity attributable to the chemotherapy. Patients had self-limited dysphagia, cough, and skin changes. Only one patient had terminal adult respiratory distress syndrome, 2 months after completion of irradiation. The cause of her pulmonary death was multifactorial, including chronic obstructive pulmonary disease and radiation fibrosis, although the final factor was acute bacterial pneumonia. Tumor recurrence was not documented in this patient, and permission for autopsy was denied. No other patient experienced clinically evident radiation pneumonitis.

DISCUSSION

Vinblastine and cisplatin administered over 3 days every 3 weeks induced an objective response in 69% (90% confidence interval of true response, 49%–86%) of the patients with advanced regional disease and in 29% (90% confidence interval of true response, 17%–47%) of the patients with metastatic non-small cell carcinoma of the lung. By dividing the cisplatin dose over 3 days, cisplatin could be given in the ambulatory care setting. Additional hydration was not needed to prevent nephrotoxicity. Due to the relatively small sample size, statistically significant conclusions relating response and such factors as histologic cell type and prior treatment in the group with metastatic disease are not feasible. However, the relative efficacy and toxicity of this chemotherapy treatment and schedule are being evaluated in patients with metastatic disease in a large phase III trial in ECOG.

For the 16 patients with advanced regional disease, the tactic of induction chemotherapy followed by irradiation proved very effective, with 81% (90% confidence interval of true response, 60%–93%) achieving a response, and did not cause additive toxicity. However, as noted, two patients died secondary to treatment, one from grade 4 leukopenia and sepsis during induction chemotherapy and the second with terminal adult respiratory distress syndrome 2 months after completion of radiation therapy. Most importantly, the overall median survival of the patients with advanced regional disease of 81 weeks compares favorably to 48 weeks in a similar group of patients treated with radiation therapy alone (14).

In designing the study there was concern about delaying the irradiation. Would the initial response to chemotherapy be high enough to justify starting with chemo-

therapy? The response to chemotherapy in the 16 patients with advanced regional disease was complete in two and partial in nine (69% response rate). This approach was further justified by the observation that for the 13 patients who received irradiation, all achieved objective response, with five complete and eight partial responses. Unfortunately, despite the high rate of response, all patients have had disease progression, including nine outside the radiation portals.

One possibility for the universal systemic progression is that maintenance chemotherapy was not used. In the initial design of this pilot study, maintenance chemotherapy was not included for several reasons. First, there was concern that chemotherapy both preceding and following irradiation might be poorly tolerated. Second, it was hoped that if induction chemotherapy were very effective, patients would have reduction in their primary tumor, experience less systemic failure, and survive longer. Third, observing relapse patterns would provide clues as to which parts of the regimen require modification. We may only speculate whether additional chemotherapy would have enhanced the response duration and survival.

Given our small sample size, no conclusions can be drawn about survival differences between this and other approaches, but a larger multi-institutional study is ongoing in ECOG to evaluate the efficacy of this regimen relative to other treatments. Other regimens with vinca alkaloids and cisplatin have shown efficacy of a variable degree (19-22). It should be noted that the original dose schedule reported by Gralla et al (1) proved quite toxic in ECOG, although the efficacy was in part confirmed (22). The regimen reported in this paper gives both agents simultaneously and apparently with less toxicity. Whether these factors influence the ultimate results cannot be stated.

In summary, our experience may nevertheless lead to

TABLE 2.—Advanced regional-disease patients: responses and characteristics

Patient No.	Cell type	Measurable or evaluable disease*	Initial performance status	RTOG T,N,M/stage	Best response to chemotherapy	Best response to irradiation	Duration of response (wks)	Survival (wks)
1	Adenocarcinoma	M	1	$T_2N_2M_0$/III	Stable disease	Partial response	42	79
2	Squamous cell carcinoma	M	1	$T_2N_2M_0$/III	Stable disease	No irradiation, died secondary to sepsis	—	8
3	Squamous cell carcinoma	M	2	$T_2N_2M_0$/III	Complete response	Complete response	76	117
4	Squamous cell carcinoma	E	1	$T_3N_2M_0$/III	Evaluable regression	Complete response	82	112
5	Squamous cell carcinoma	E	1	$T_3N_2M_0$/III	Evaluable regression	Complete response	73	81
6	Adenocarcinoma	M	1	$T_3N_2M_0$/III	Partial response	Partial response	23	39
7	Large cell carcinoma	M	1	$T_3N_2M_1$/IV	Partial response	Partial response	26	37
8	Adenocarcinoma	M	1	$T_1N_2M_0$/III	Stable disease	Partial response	25	37
9	Adenocarcinoma	M	1	$T_2N_2M_0$/III	Partial response	Partial response	31	131
10	Squamous cell carcinoma	E	2	$T_2N_2M_0$/III	Evaluable regression	Evaluable regression	21	60
11	Adenocarcinoma	M	1	$T_2N_2M_0$/III	Stable disease	No irradiation, died secondary to unrelated factors	6	6
12	Squamous cell carcinoma	E	1	$T_3N_1M_0$/III	Evaluable regression	Evaluable regression	29	29
13	Adenocarcinoma	E	2	$T_2N_2M_0$/III	Stable disease	No irradiation, progression during induction chemotherapy	4	36
14	Adenocarcinoma	E	1	$T_2N_2M_0$/III	Evaluable regression	Evaluable regression†	39	91
15	Adenocarcinoma	E	1	$T_2N_2M_0$/III	Evaluable regression	Complete response	81	100
16	Squamous cell carcinoma	E	1	$T_2N_2M_0$/III	Complete response	Complete response	81	143

*M = measurable disease; E = evaluable disease.
†Based on decrease in pleural effusion.

the following conclusions. (*a*) The dose schedule of cisplatin and vinblastine as indicated has reproducible activity in non-small cell lung cancer. (*b*) With this regimen, the response and median survival for patients with advanced regional disease are superior to those of patients with metastatic disease. Of note, only one patient in the group with advanced regional disease progressed during induction chemotherapy. (*c*) The strategy of beginning patients with advanced regional disease with an active and well-tolerated chemotherapy regimen followed by consolidation with irradiation has yielded encouraging response rates and improved survival compared to a similar group receiving irradiation alone. This concept must be tested further.

REFERENCES

1. GRALLA RJ, CASPER ES, KELSEN DP, ET AL. Cisplatin and vindesine for advanced carcinoma of the lung: randomized trial investigation of two dose schedules. Ann Intern Med 92:414-420, 1981.
2. GRALLA RJ, RAPHAEL BG, GOLBEY RB, ET AL. Phase II evaluation of vindesine in patients with non-small cell carcinoma of the lung. Cancer Treat Rep 63:1343-1346, 1979.
3. FURNAS B, EINHORN LH, and ROHN RJ. A phase II trial of vindesine in non-small cell lung cancer. Proc Am Assoc Cancer Res and ASCO 21:448, 1980.
4. GRUNDTVIG P, OSTERLIND K, PEDERSEN AG, ET AL. Phase II trial of vindesine in bronchogenic carcinoma of W.H.O. types I, III, and IV. *In* Abstracts of Second World Conference on Lung Cancer (Hansen HH, and Dombernowsky P, eds). Amsterdam, Excerpta Medica, 1980.
5. MATTSON K, HOLSTI LR, SALMO M, ET AL. Vindesine in the treatment of small cell and non-small cell bronchogenic carcinoma: preliminary results. Cancer Treat Rev 7(suppl):65-70, 1980.
6. LUEDKE D, LUEDKE S, PETRUSKA P, ET AL. A randomized prospective study of vindesine vs doxorubicin and cyclophosphamide in the treatment of advanced squamous cell carcinoma of the lung. Proc Am Assoc Cancer Res and ASCO 22:499, 1981.
7. DEJAGER R, LONGEVAL E, and KLASTERSKY J. High-dose cisplatin with fluid and mannitol-induced diuresis in advanced lung cancer: a phase II clinical trial of the EORTC Lung Cancer Working Party (Belgium). Cancer Treat Rep 64:1341-1346, 1980.
8. ROSOFF AH, BEARDON JD, III, and COLTMAN CA, JR. Phase II evaluation of *cis*-diamminedichloroplatinum(II) in lung cancer. Cancer Treat Rep 60:1679-1680, 1976.
9. CASPER ES, GRALLA RJ, KELSEN DP, ET AL. Phase II study of high-dose *cis*-dichlorodiammineplatinum(II) in the treatment of non-small cell lung cancer. Cancer Treat Rep 63:2107-2109, 1979.
10. BRITELL JC, EAGAN RT, INGLE JN, ET AL. *cis*-Dichlorodiammineplatinum(II) alone followed by adriamycin plus cyclophosphamide at progression versus *cis*-dichlorodiammineplatinum(II), adriamycin, and cyclophosphamide in combination for adenocarcinoma of the lung. Cancer Treat Rep 62:1207-1210, 1978.
11. BERENZWEIG M, VOGL SE, KAPLAN BH, ET AL. Phase II trial of *cis*-diamminedichloroplatinum in patients with non-small cell bronchogenic carcinoma not exposed to prior chemotherapy. Proc Am Assoc Cancer Res and ASCO 21:457, 1980.
12. CREASEY WA. Vinca Alkaloids. Cancer Chemotherapy III. The Forty-Sixth Hahnemann Symposium (Brodsky I, Kahn SB, and Conroy JF, eds). New York, Grune and Stratton, 1978, p 493.
13. MUGGIA FM, BLUM RH, and FORMAN JD. Role of chemotherapy in the treatment of lung cancer: evolving strategies for non-small cell histologies. Int J Radiat Oncol Biol Phys 10:137-145, 1984.
14. PEREZ CA, STANLEY K, MIETLOWSKI T, ET AL. Radiation therapy in the treatment of non-small cell bronchogenic carcinoma—preliminary results of two dose fractionation studies. *In* Lung Cancer: Progress in Therapeutic Research (Muggia FM, and Rozencweig M, eds). New York, Raven Press, 1979.
15. FREI E. Multidisciplinary treatment for cancer. New opportunities. Int J Radiat Oncol Biol Phys 8:951-952, 1982.
16. EAGAN RT, FLEMING TR, and SCHOONOVER V. Evaluation of response criteria in advanced lung cancer. Cancer 44:1125-1128, 1979.
17. OKEN MM, CREECH RH, TORMEY DC, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.
18. RUBIN P, PEREZ CA, and KELLER B. The logical basis of radiation treatment policies in the multidisciplinary approach to lung cancer. *In* Lung Cancer: Natural History, Prognosis, and Therapy (Israel L, and Chahinian AP, eds). New York, Academic Press, 1976.
19. KALMAN LA, KRIS MG, GRALLA RJ, ET AL. Vinca alkaloid and cisplatin combination chemotherapy in non-small cell cancer: results of randomized trial with the comparison of methods of response assessment in 109 patients. Proc ASCO 2:201, 1983.
20. WOODCOCK TM, ALLEGRA JC, RICHMAN SP, ET AL. Vinblastine (VLB) and cisplatin (DDP) in non-small cell lung cancer (NSCLC). Proc ASCO 1:154, 1982.
21. STOOPLER MG, JARETZKI A, RAKOWSKI TJ, ET AL. Vinblastine (VLB) and *cis*-dichlorodiammineplatinum (DDP) combination chemotherapy in non-small cell lung cancer (NSCLC). Proc ASCO 1:141, 1982.
22. RUCKDESCHEL JC, FINKELSTEIN DM, and ETTINGER DS. Chemotherapy of metastatic non-small cell bronchogenic carcinoma (NSCBC): a randomized comparison of the four most active regimens. Proc Am Assoc Cancer Res 25:171, 1984.

Combination Chemotherapy With Vindesine, Etoposide, and Cisplatin in Non-Small Cell Lung Cancer: A Pilot Study of the Southeastern Cancer Study Group[1,2]

John D. Hainsworth,* Lester L. Porter, III, David H. Johnson, Kenneth R. Hande, Steven N. Wolff, Robert Birch, Gregory Enas, and F. Anthony Greco[3]

Ninety-two patients with advanced non-small cell lung cancer were treated with a combination chemotherapy regimen containing cisplatin, vindesine, and etoposide. Eighteen patients (20%) achieved major responses to therapy (three complete responders and 15 partial responders). Response rates were similar in each histologic subtype. Initial performance status was an important determinant of response; 42% of the patients with a Karnofsky performance status ≥ 70% responded versus 5% of those with a performance status < 50%. The median duration of partial response was 21 weeks; complete responders had a median response duration of 50 weeks. The median survival for the entire group was 23.5 weeks. Toxicity with this regimen was acceptable; myelosuppression was the major toxic effect and was severe in only 10% of the patients. This regimen produced response rates comparable to those reported with other combinations containing cisplatin. Survival advantage in patients receiving this treatment is not established. [Cancer Treat Rep 70:339–341, 1986]

Non-small cell carcinoma of the lung remains a chemotherapy-resistant malignancy. Recently, the experimental vinca alkaloid vindesine has demonstrated significant activity against advanced non-small cell lung cancer, both as a single agent and in combination with cisplatin (1,2). Although etoposide has shown limited activity as a single agent in non-small cell lung cancer, its use in combination with cisplatin may be synergistic against small cell lung cancer and germinal tumors (3,4). In addition, some encouraging results in non-small cell lung cancer have been reported using cisplatin and etoposide in combination (5).

We report here the results of a pilot study of the Southeastern Cancer Study Group evaluating the toxicity and response rate of a three-drug combination regimen using vindesine, etoposide, and cisplatin in the treatment of non-small cell lung cancer.

PATIENTS AND METHODS

Between June 1981 and November 1983, 95 patients with biopsy-proven non-small cell lung cancer were entered in this study. Patients were stratified by histologic subtype (squamous carcinoma, adenocarcinoma, and large cell carcinoma); 30 patients with each histology were targeted for accession to this study. Three patients were subsequently found to be ineligible and are excluded. The clinical characteristics of the 92 patients who received treatment are summarized in table 1. The median Karnofsky performance status was 60% at the time of initiation of chemotherapy; 19 patients had a Karnofsky performance status < 50%. The unusual distribution of histologies is explained by the fact that accrual of each histology was stopped as the number of patients approximated 30. All patients had either metastatic or locally unresectable carcinoma. Most patients (84%) were previously untreated; no patient had received previous chemotherapy with any of the drugs used in this study.

The chemotherapy induction regimen is shown in table 2. Following 6 weeks of induction therapy, patients were evaluated for response at Week 8. Patients with complete response, partial response, or stable disease (all response criteria defined in the usual manner) continued with maintenance doses of all three drugs (cisplatin, 60 mg/m^2; vindesine, 3 mg/m^2; and

[1]Received Apr 8, 1985; revised June 10, 1985; accepted June 13, 1985.

[2]Supported in part by Public Health Service grants CA-19429, CA-23909, CA-19657, and CA-24456 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; and by Lilly Research Laboratories.

[3]Division of Oncology, Vanderbilt University School of Medicine, Nashville, TN; and the Southeastern Cancer Study Group Statistical Center, University of Alabama, Birmingham.

Reprint requests to: John D. Hainsworth, MD, Division of Oncology, Vanderbilt University Medical Center, Nashville, TN 37232.

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Median age in yrs (range): 57 (38-72)	
Males/females	70/22
Performance status (Karnofsky)	
< 50%	19 (21)
50%-60%	47 (51)
⩾ 70%	26 (28)
Prior treatment status	
No prior treatment	77 (84)
Radiation therapy only	13 (14)
Chemotherapy only	2 (2)
Histologic type	
Adenocarcinoma	36 (40)
Epidermoid carcinoma	28 (30)
Large cell undifferentiated carcinoma	28 (30)
Extent of disease	
Locally unresectable	23 (25)
Distant metastases	69 (75)

etoposide, 100 mg/m^2) at 28-day intervals until progression occurred. Dosage reductions were made during the induction phase of therapy for myelosuppression: wbc count 3000-4000/mm^3, 75% dose; wbc count 2000-3000/mm^3, 50% dose; and wbc count < 2000/mm^3, dosage withheld.

RESULTS

Seventy-one of 92 patients (77%) completed the 6-week course of induction therapy and were evaluable for response at Week 8. Twenty-one patients (23%) were unable to complete the induction course of therapy due to disease progression and declining performance status; these patients are considered treatment failures.

Toxicity during the 6-week induction course was mild to moderate. Myelosuppression was the major toxic effect, with wbc count nadirs < 1000/mm^3 in ten patients (11%). Six patients were hospitalized with infections while neutropenic. Only one patient (1%) experienced a platelet count nadir < 50,000/mm^3 and required platelet transfusion. Twelve patients (13%) developed severe anemia (hematocrit < 25%) during induction therapy. Forty-two patients (46%) developed peripheral neuropathy; however, this was severe enough to impair function in only three patients (4%). Nausea and vomiting were mild to moderate, with no patient requiring hospitalization for these side effects. Nine patients had mild increases in serum creatinine, but in only one patient did the serum creatinine rise to > 2.0 mg/dl. The monthly maintenance therapy was generally well-tolerated, with only two patients experiencing severe myelosuppression (wbc count < 1000/mm^3).

Eighteen of 92 patients (20%; 95% confidence limits, 12%-29%) had major responses to therapy [complete response, three patients (3%); partial response, 15 patients (16%)]. An additional 29 patients (32%) had minimal response or stable tumor measurements following induction therapy. Major responses were seen with comparable frequency in each of the histologic subtypes (squamous carcinoma, 21%; adenocarcinoma, 14%; and large cell carcinoma, 25%). Eleven of 26 patients (42%) with a Karnofsky performance status ⩾ 70% had response. In contrast, only one of 19 patients (5%) with an initial Karnofsky performance status < 50% achieved partial response; 12 of these 19 patients (63%) were withdrawn from the study before reaching the 8-week evaluation period. Response rates were similar in previously untreated and previously treated patients.

TABLE 2.—Six-wk induction regimen using cisplatin, vindesine, and etoposide

Drug	Dose (mg/m^2 iv)	Week— 1	2	3	4	5	6	7	8*
Cisplatin	60	X				X			
Vindesine	3.0	X	X	X	X	X	X	X	
Etoposide	100	X	X			X	X		

* Evaluation was on Week 8.

Figure 1 is a survival curve for the entire group. Median survival was 23.5 weeks. Disease progressed in all patients. The median response duration was 50 weeks for complete responders and 21 weeks for partial responders.

DISCUSSION

The results with this three-drug combination regimen in non-small cell lung cancer are comparable to results reported with several other regimens (2,5-7). Seventeen of 18 responses occurred in patients with good performance status and were generally of brief dura-

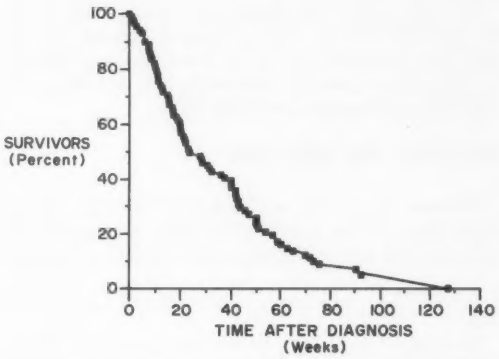

FIGURE 1.—Survival curve for 92 patients demonstrating median survival of 23.5 wks.

tion. No patient has experienced a long disease-free survival.

Toxicity using this regimen was tolerable, and no unexpected toxic effects occurred as a result of the continued use of these three agents. Myelosuppression was the major toxic effect encountered, but was severe in only a small percentage of patients.

Although treatment results with this regimen are comparable to those with other cisplatin-containing regimens, we cannot recommend its routine use in patients with advanced non-small cell lung cancer. The efficacy of this and other chemotherapy regimens in prolonging survival in non-small cell lung cancer remains unproven.

REFERENCES

1. GRALLA RJ, RAPHAEL BG, GOLBEY RB, ET AL. Phase II evaluation of vindesine in patients with non-small cell carcinoma of the lung. Cancer Treat Rep 63:1343-1346, 1979.
2. GRALLA RJ, CASPER ES, KELSEN DP, ET AL. Cisplatin and vindesine combination chemotherapy for advanced carcinoma of the lung: a randomized trial investigating two dosage schedules. Ann Intern Med 95:414-420, 1981.
3. PORTER L, JOHNSON D, HAINSWORTH J, ET AL. Recurrent small cell lung carcinoma (SCLC) treated with VP-16 and cisplatinum. Proc ASCO 3:215, 1984.
4. HAINSWORTH JD, GRECO FA, WILLIAMS SD, ET AL. VP-16 in the treatment of refractory germinal neoplasms. *In* Etoposide (VP-16) (Issel B, et al, eds). New York, Academic Press, Inc, 1984, pp 243-252.
5. LONGEVAL E, and KLASTERSKY J. Combination chemotherapy with cisplatin and etoposide in bronchogenic squamous cell carcinoma and adenocarcinoma. Cancer 50:2751-2756, 1982.
6. NEILL HB, GRIFFIN JP, WEST WH, ET AL. Combination chemotherapy with mitomycin C, methotrexate, cisplatin, and vinblastine in the treatment of non-small cell lung cancer. Cancer 54:1260-1263, 1984.
7. WOODCOCK TM, BLUMENREICH MS, RICHMAN SP, ET AL. Combination chemotherapy with *cis*-diamminedichloroplatinum and vinblastine in advanced non-small cell lung cancer. J Clin Oncol 1:247-250, 1983.

Salvage Chemotherapy in Hodgkin's Disease Irradiation Failures: Superiority of Doxorubicin-Containing Regimens Over MOPP[1]

A. Santoro,[2,*] S. Viviani,[2] C. J. R. Villarreal,[2,3] V. Bonfante,[2] A. Delfino,[2] P. Valagussa,[2] and G. Bonadonna[2]

One hundred and twenty-two consecutive patients with Hodgkin's disease who relapsed after primary curative irradiation were treated with either MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) or a doxorubicin-containing regimen (ADM-Reg). The main pretreatment characteristics were comparable in the two groups. Complete remission was achieved in 74.6% of patients treated with MOPP (44 of 59) and in 90.5% of those given ADM-Reg (57 of 63). No difference was observed in the incidence of complete remission with regard to the type of ADM-Reg utilized [MABOP (mechlorethamine, doxorubicin, bleomycin, vincristine, and prednisone), 92.9%; ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine), 95.6%; and MOPP alternated with ABVD, 84.6%]. The superiority of ADM-Reg versus MOPP was also confirmed in the 7-year analysis of freedom from disease progression (73.2% vs 42.2%), relapse-free survival (81.2% vs 54.3%), and overall survival (80.5% vs 44.4%). Thrombocytopenia was less frequently observed with ADM-Reg (30%), particularly following ABVD (13%), compared to MOPP (73%). The lowest incidence of alopecia occurred in patients given MOPP (15%) or MOPP/ABVD (19%). Acute nonlymphoblastic leukemia was observed in patients treated with MOPP (five of 59) and MABOP (one of 14). The observed findings indicate that in patients failing to respond to primary radiotherapy, salvage regimens containing doxorubicin are more effective than MOPP. Furthermore, combinations devoid of procarbazine and alkylating agents (ABVD) or with less intensive administration of these drugs (MOPP/ABVD) were not associated with secondary leukemia.
[Cancer Treat Rep 70:343–348, 1986]

The treatment for all stages of Hodgkin's disease has improved dramatically during the past 15 years with the advent of accurate staging, intensive radiotherapy, and cyclic combination chemotherapy (1–3). Nevertheless, a significant fraction of patients, ranging from 20% to 50%, treated with primary radiotherapy for stages I, II, and IIIA will relapse, especially within the first 3 years from completion of irradiation (1,3–5). In toto, the available data suggest that patients who relapse do not fare as well with re-irradiation as patients with an equivalent extension of primary tumor (6,7). By contrast, several reports have clearly shown that previous radiotherapy does not adversely affect both incidence and duration of complete remission (CR) with subsequent chemotherapy. In fact, MOPP (mechlorethamine, vincristine, procarbazine, and prednisone) or MOPP-like regimens induce CR in 62%–91% of patients relapsing from irradiation, with a long-term disease-free survival ranging from 45% to 80% (8–13).

By the end of 1972, following a phase II study with doxorubicin and bleomycin (14,15), our research group decided to include these two agents in a multidrug combination for advanced Hodgkin's disease. The first regimen utilized, MABOP (mechlorethamine, doxorubicin, bleomycin, vincristine, and prednisone), failed (as did other MOPP-derived combinations) to improve the incidence and the duration of CR over MOPP (16). However, this early experience contributed to the development in 1973 of the ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) regimen (17,18). Through prospective randomized studies, ABVD proved to be as effective, and in given subsets, more effective, than MOPP in previously untreated patients with advanced Hodgkin's disease (17–20). Subsequently, our strategy to alternate one cy-

[1]Received Sept 20, 1985; revised Dec 3, 1985; accepted Dec 10, 1985.
[2]Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy.
[3]*Present address:* Monterrey Hospital, Mexico.
[*]*Reprint requests to:* Dr. Armando Santoro, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venezian, 1, 20133 Milan, Italy.

cle of MOPP with one cycle of ABVD resulted in a higher incidence of CR, relapse-free, and total survival rates in stage IV Hodgkin's disease compared to MOPP alone (19,21,22). The results of the above-mentioned trials not only demonstrated that the cyclic delivery of highly effective non-cross-resistant combinations can be superior to a single polydrug regimen, but also emphasized the therapeutic usefulness of regimens containing doxorubicin, a drug not previously included in combinations for the treatment of Hodgkin's disease.

The aim of this study was to retrospectively analyze the therapeutic results achieved with combination chemotherapy in patients failing to respond to primary irradiation and to compare the efficacy of doxorubicin-containing regimens (ADM-Reg) versus MOPP.

PATIENTS AND METHODS

Between February 1971 and February 1983, 122 consecutive patients with biopsy-proven diagnosis of Hodgkin's disease who had relapsed after primary radiotherapy for stage I (A + B), II (A + B), and IIIA were treated with combination chemotherapy. Fifty-nine patients received MOPP and 63 received an ADM-Reg (MABOP, 14 patients; ABVD, 23; and MOPP alternated monthly with ABVD, 26). The comparative pretreatment characteristics are shown in table 1. The median age was 35 years in the MOPP group (range, 15–62) and 34 years in ADM-Reg group (range, 17–70). The two groups were comparable as far as the main prognostic variables were concerned. However, nodular sclerosis histology was comparatively more frequent in the MOPP group (59% vs 48%), while distant extranodal involvement was more often observed in the group treated with ADM-Reg (57% vs 47%). The percent differences were not significant. In a total of 18 patients (MOPP, seven; ADM-Reg, 11), the initial chest x-rays were not available for an objective reevaluation of bulky lymphoma, ie, mass thoracic ratio > 0.33 (23).

In addition to physical examination, the extent of recurrent disease was reassessed in all patients by chest roentgenogram, lymphography, and two needle bone marrow core biopsies from the posterior iliac crest. Restaging through laparoscopy with multiple liver biopsies was carried out in two-thirds of the patients. The median time from the end of primary radiotherapy to the start of salvage chemotherapy was 24 months (range, 0–183) in the MOPP group and 21 months (range, 0–155) in the ADM-Reg group, respectively.

Our strategic approach to primary irradiation failures has varied over the years from chemotherapy sequentially combined with different extent and timing of irradiation to chemotherapy alone. In 78 of 122 (64%) patients (MOPP, 38; ADM-Reg, 40), salvage combination chemotherapy was administered alone once irradiation failure was documented. The drug regimens utilized were delivered according to the classical dose schedules previously described (2,19). When a single polydrug therapy (MABOP, MOPP, or ABVD) was utilized, a minimum of six treatment cycles were planned. For patients entered in the trial randomizing MOPP (12 patients) versus MOPP/ABVD (13 patients), as well as for all patients treated with the alternating regimen, a total of 12 monthly cycles were planned (19). In nine (7%) patients (MOPP, eight; ADM-Reg, one), chemotherapy was delivered almost simultaneously with irradiation, which was limited to the single most important nodal site. In 11 (9%) patients (MOPP, six; ADM-Reg, five), three to six cycles of either MOPP or ABVD were administered before and after involved-field radiotherapy. In 24 (20%) patients (MOPP, seven; MABOP or ABVD, 17), six chemotherapy cycles were followed by involved-field irradiation as consolidation treatment. In the above-mentioned three groups, the dose of high-energy irradiation varied from 3000 to 4000 rad. In all patients started on salvage chemotherapy, the maximum cumulative dose of doxorubicin was 575 mg/m^2 and the maximum cumulative dose of bleomycin was 240 units/m^2.

Patients were reassessed 1 month following the com-

TABLE 1.—Comparative pretreatment characteristics before starting combination chemotherapy*

Characteristic	MOPP (n = 59)	ADM-Reg (n = 63)
Sex		
Males	34 (58)	36 (57)
Females	25 (42)	27 (43)
Age (yrs)		
≤ 40	40 (68)	43 (68)
> 40	19 (32)	20 (32)
Histology		
Nodular sclerosis	35 (59)	30 (48)
Other histologies	24 (41)	33 (52)
Extent of disease		
Nodal only	31 (52.5)	27 (43)
Extranodal ± nodal	28 (47)	36 (57)
1 extranodal site	21/28 (75)	30/36 (83)
> 1 extranodal site	7/28 (25)	6/36 (17)
Systemic symptoms		
No	34 (58)	38 (60)
Yes	25 (42)	25 (40)
Bulky disease		
Yes	13 (22)	12 (19)
No	39 (66)	40 (63.5)
Unknown	7 (12)	11 (17.5)
Extent of previous RT		
Involved field	13 (22)	13 (21)
Mantle	18 (31)	19 (30)
Subtotal nodal	26 (44)	26 (41)
Total nodal	2 (3)	5 (8)
Median time in mos from RT to CT (range)	24 (0–183)	21 (0–155)
Median follow-up in mos from start of CT (range)	89 (32–125)	81 (10–154)

*RT = radiotherapy; CT = chemotherapy. Unless otherwise specified, values = No. of patients (%).

pletion of the treatment plan. We repeated all tests in which findings had been positive at the start of chemotherapy, including re-biopsy of both liver and bone marrow if these organs were known to be previously involved by Hodgkin's disease. A patient was considered to be in CR when all signs and symptoms of disease had disappeared for at least 1 month. Partial remission (PR) was defined as a > 50% reduction in the product of the longest perpendicular diameters of all sites of measurable disease. Patients with tumor regression of < 50% were designated as nonresponders.

Degree of statistical significance on fractions of complete responders versus nonresponders was calculated by the chi-square test. Remission duration and overall survival were computed from the day chemotherapy was started, according to the life-table method. Degree of statistical significance of observed difference in various subgroups was assessed with the log-rank test (24). The percentage of patients in remission or surviving is reported in the text for one time point, whereas the P values represent comparison of the entire plots.

RESULTS

Table 2 compares the remission rate between the two treatment groups. CR was achieved in 74.6% of patients treated with MOPP (44 of 59) and in 90.5% of all patients given an ADM-Reg (57 of 63), while PR was documented in 8.5% and 1.6% of patients, respectively. The difference in the CR rate was significant ($P < 0.04$). In patients treated with chemotherapy alone, CR was 66% following MOPP and 85% after ADM-Reg, with no particular difference regarding the type of combination utilized (MABOP, 80%; ABVD, 88.9% and MOPP/ABVD, 84.6%). The median number of treatment cycles required to document complete tumor regression was five in the MOPP group and four in the ADM-Reg group.

When the sandwich approach was utilized, CR was achieved before radiotherapy in three of six patients in the MOPP group and in three of five patients in the ADM-Reg group, respectively. In the subgroup where radiotherapy was delivered as consolidation, all patients achieved CR before irradiation, with the exception of one patient in the MOPP group who had minimal residual disease following six cycles of chemotherapy. Considering all patients given an ADM-Reg, the CR rate was as follows: MABOP, 92.9%; ABVD, 95.6%; and MOPP/ABVD, 84.6%.

Table 3 shows the comparative incidence of CR related to the pretreatment characteristics. The likelihood of achieving CR in the MOPP group was influenced by the extent of disease (extranodal lymphoma, 60.7%; nodal lymphoma, 87.1%; $P < 0.05$) and by the presence of systemic symptoms (symptoms, 60%; no symptoms, 85.3%; $P = 0.09$). In contrast, in the ADM-Reg group, there was no evidence between a complete tumor response and any of the patient characteristics. The comparative analysis of results presented in table 3 indicated that the percent CR rate was superior in the ADM-Reg group versus the MOPP group for all subgroups, except for the small subset presenting with bulky disease. However, because of the limited number of patients in each prognostic category, differences in the percentage of patients with CR between the two treatment groups failed to achieve statistical significance, with the exception of the patient subset presenting with systemic symptoms ($P < 0.03$).

Figure 1 displays the comparative probability of freedom from disease progression. Forty-two percent of patients given MOPP showed progressive lymphoma within 2 years from the start of chemotherapy compared to only 18% of those treated with ADM-Reg. At 7 years, 42.2% of the original 59 patients in the MOPP group remained free of disease progression compared to 73.2% of the 63

TABLE 2.—Comparative CR related to treatment subgroups*

Treatment subgroup	No. of patients/total (%)	
	MOPP	ADM-Reg
CT	25/38 (66)	34/40 (85.0)
RT + CT	6/8 (75)	1/1 (100)
CT→RT→CT	6/6 (100)	5/5 (100)
CT→RT	7/7 (100)	17/17 (100)
All patients	44/59 (74.6)	57/63 (90.5)†

*CT = chemotherapy; RT = radiotherapy.
†$P < 0.04$.

TABLE 3.—Comparative incidence of CR related to pretreatment characteristics

Characteristic	No. of patients (%)	
	MOPP	ADM-Reg
Sex		
Males	24 (70.6)	32 (88.9)
Females	20 (80.0)	25 (92.6)
Age (yrs)		
≤ 40	31 (77.5)	39 (90.7)
> 40	13 (68.4)	18 (90.0)
Histology		
Nodular sclerosis	26 (74.3)	27 (90.0)
Other histologies	18 (75.0)	30 (90.9)
Extent of disease		
Nodal	27 (87.1)	26 (96.3)
Extranodal	17 (60.7)	31 (86.1)
Systemic symptoms		
No	29 (85.3)	34 (89.5)
Yes	15 (60.0)*	23 (92.0)*
Bulky disease		
Yes	10 (76.9)	8 (66.7)
No	28 (71.8)	39 (97.5)
Unknown	6 (85.7)	10 (90.9)
Free interval from end of radiotherapy (yrs)		
≤ 3	29 (72.5)	39 (88.6)
> 3	15 (78.9)	18 (94.7)

*$P < 0.03$.

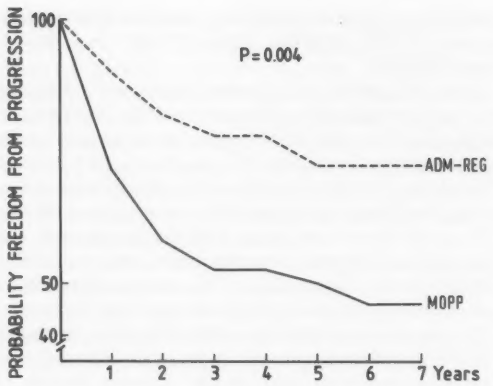

FIGURE 1.—Probability of remaining free of disease progression.

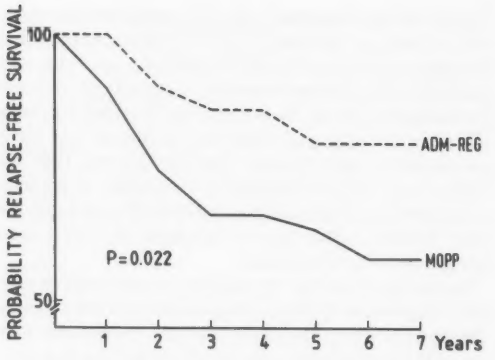

FIGURE 2.—Relapse-free survival probability in patients who achieved CR.

patients given ADM-Reg ($P = 0.004$). A significant difference favoring ADM-Reg versus MOPP (fig 2) was also detected in the 7-year relapse-free survival (RFS) for patients who achieved CR (MOPP, 54.3% vs ADM-Reg, 81.2%; $P = 0.022$). There was no significant difference in the RFS among the three ADM-Reg. In both treatment groups, the analysis of RFS failed to demonstrate any advantage by the delivery of consolidation radiotherapy following six cycles of chemotherapy (MOPP: radiotherapy, 57.1%; no radiotherapy, 52.8%; ADM-Reg: radiotherapy, 81.1%; no radiotherapy, 80.9%). As shown in figure 3, significant increase in the overall survival (OS) was obtained with ADM-Reg over MOPP. At 7 years from starting chemotherapy, 44.4% of 59 patients in the MOPP group and 80.5% of 63 patients in the ADM-Reg group remained alive ($P = 0.0054$).

Table 4 summarizes the major early toxic manifestations related to the drug regimens utilized. The most relevant difference concerns the low incidence of thrombocytopenia in the ABVD group (13%) compared to all three regimens containing procarbazine. In particular, none of the 23 patients treated with ABVD alone showed a platelet fall to $< 50{,}000/mm^3$ compared to MOPP (12.5%), MABOP (14.3%), and MOPP/ABVD (7.7%). Seventy-nine percent of patients treated with ADM-Reg received > 75% of the planned dose of doxorubicin and vinblastine compared with 65% of mechlorethamine and procarbazine in MOPP-treated patients. Paresthesias were less frequently observed following vinblastine compared to vincristine-containing regimens. All regimens, including doxorubicin, produced more frequent severe vomiting compared to MOPP. Despite the fact that all patients had been previously irradiated to the mediastinal area, no patients showed symptoms or signs indicative of cardiomyopathy from doxorubicin or lung fibrosis from bleomycin. Six patients developed acute nonlymphoblastic leukemia when chemotherapy included mechlorethamine with or without procarbazine (MOPP, five; MABOP, one). The median time from beginning of chemotherapy to the diagnosis of leukemia was 49 months (range, 35-65). In addition, two patients developed solid tumors, 94 months from the start of MOPP (epidermoid carcinoma of the tongue) and 72 months from MOPP alternated with ABVD (adenocarcinoma of the colon), respectively.

DISCUSSION

During the past 15 years, the general philosophy in the

TABLE 4.—Comparative toxicity*

		ADM-Reg			
Toxic effect	MOPP	Total	ABVD	MOPP/ ABVD	MABOP
Leukopenia†	89	75	78	81	79
Thrombocytopenia‡	73	30	13	50	29
Complete alopecia	15	33	43	19	43
Paresthesias	68	44	33	54	42
Adynamic ileus	12	5	5	4	8

*Values = % of patients.
†Leukocyte count $< 3800/mm^3$.
‡Platelet count $< 120{,}000/mm^3$.

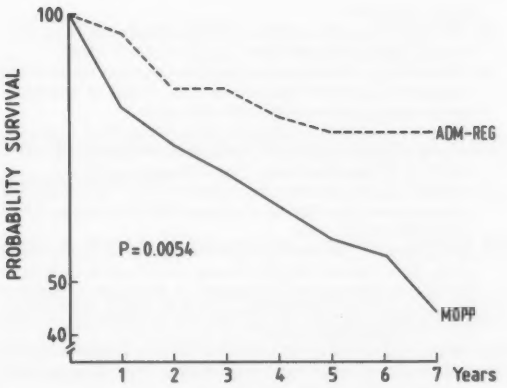

FIGURE 3.—Overall survival probability from the start of chemotherapy.

management of patients with early-stage Hodgkin's disease consisted of maximizing staging to minimize therapy and thereby minimize significant long-term complications (1,3,5). Nevertheless, a number of reports (5,25,26,27) have indicated that staging laparotomy should not be used routinely, even in patients with early stages, because of the comparable results between laparotomy and nonlaparotomy patients in terms of freedom from disease progression and survival. In addition, despite accurate surgical staging and modern radiation techniques, 20%-50% of patients with stage I, II, and IIIA will eventually relapse and therefore will require further therapy (1,3,5,28). Attempts have been made to identify patients with poor prognostic characteristics, such as the presence of systemic "B" symptoms, involvement of more than three nodal sites, as well as bulky disease in the mediastinum for which primary combined radiotherapy-chemotherapy approach is advisable (1,3,5,23).

A major problem is represented by the optimal management of patients failing to respond to primary extensive radiotherapy. In fact, it is well known that the response to MOPP or "MOPP-like" chemotherapy is not compromised by prior exposure to radiotherapy (11). As shown in table 5, in patients failing to respond to primary irradiation, the average CR rate following MOPP ranged from 75% to 91%, with RFS and OS rates ranging from 45% to 69% and 44% to 73%, respectively. Comparable results were achieved by other research groups utilizing MOPP-derived regimens alone (30,31) or MOPP combined with radiotherapy (13).

The results of our retrospective study with salvage chemotherapy in irradiation failures showed that all three ADM-Reg utilized yielded higher CR, RFS, and OS rates compared to MOPP. Our findings with MOPP are comparable to those reported by other research groups, as summarized in table 5. In our own experience, the superiority of ADM-Reg over MOPP was not limited to this retrospective series of patients. In fact, other prospective randomized trials have indicated that ABVD consistently yielded superior results compared to MOPP when either regimen was combined with irradiation in stages IIB, IIIA, and IIIB (18,19,20) or when ABVD was alternated monthly with MOPP in stage IV (21,22).

There are two possible reasons to explain present findings. One may be related to the superior activity of given drugs included in the ABVD regimen. Prior experience has shown that in Hodgkin's disease vinblastine is more effective than vincristine. Furthermore, phase II studies have indicated that the response rate following treatment with doxorubicin ranges from 40% to 70% of patients with Hodgkin's disease refractory to alkylating agents, procarbazine, and vinca alkaloids (14,32,33). Therefore, we may assume that this anthracycline derivative is superior to mechlorethamine or procarbazine. The second reason is the demonstrated superiority of alternating non-cross-resistant regimens, such as MOPP/ABVD, versus MOPP alone (21,22).

In conclusion, present findings would suggest that an ADM-Reg should be the preferred drug treatment to be utilized as salvage chemotherapy in irradiation failures. The advantage of such treatment would include a decreased risk of leukemogenesis (34,35) and irreversible gonadal dysfunction (19,36) when alkylating agents and procarbazine are either deleted or administered through a less intensive schedule in previously irradiated patients. Our 7-year results (20) from a randomized study

TABLE 5.—Salvage chemotherapy in patients with Hodgkin's disease relapsing after radiotherapy

Author (ref No.)	Regimen	No. of patients	Results*		
			CR	RFS	Survival
Canellos et al (8)	MOPP	21	76	Unknown	75 (3)
Portlock et al (9)	MOPP	46	78	45 (5)	58 (5)
DeVita et al (11)	MOPP	32	91	69 (5)	73 (5)
Mauch et al (12)	MOPP	58	Unknown	55 (5)	60 (4)
Cooper et al (29)	MOPP	34	62	Unknown	Unknown
Bakemeier et al (30)	MOPP	86	70	44 (5)	Unknown
Present study	MOPP	59	75	54 (7)	44 (7)
	ADM-Reg	63	90	81 (7)	80 (7)

* Values are in %; values in parentheses = yrs at risk.

testing MOPP versus ABVD within a combined modality approach have confirmed the lack of cardiac toxicity related to doxorubicin and of important lung toxicity from bleomycin, respectively, in patients irradiated to the mediastinum.

REFERENCES

1. KAPLAN HS. Hodgkin's Disease. Cambridge, MA, Harvard University, 1980.
2. DEVITA VT. The consequence of the chemotherapy of Hodgkin's disease. The 10th David A. Karnofsky Memorial Lecture. Cancer 47:1-13, 1981.
3. BONADONNA G, and SANTORO A. Evolution in the treatment strategy of Hodgkin's disease. Adv Cancer Res 36:257-293, 1982.
4. WELLER SA, GLATSTEIN E, KAPLAN HS, ET AL. Initial relapse in previously treated Hodgkin's disease. I. Results of second treatment. Cancer 37:2840-2846, 1976.
5. HOPPE RT. Stage I-II Hodgkin's disease: current therapeutic options and recommendations. Blood 62:32-36, 1983.
6. WELLER SA, GLATSTEIN E, CASTELLINO RA, ET AL. Initial relapse in previously treated Hodgkin's disease. II. Retrograde transdiaphragmatic extension. Int J Radiat Oncol Biol Phys 2:863-872, 1977.
7. DEVITA VT. Hodgkin's disease: the salvage of radiation treatment failures. Int J Radiat Oncol Biol Phys 2:1035-1037, 1977.
8. CANELLOS GP, YOUNG RC, and DEVITA VT. Combination chemotherapy for advanced Hodgkin's disease in relapse following extensive radiotherapy. Clin Pharmacol Ther 13:750-754, 1972.
9. PORTLOCK CS, ROSENBERG SA, GLATSTEIN E, ET AL. Impact of salvage treatment on initial relapses in patients with Hodgkin's disease, stages I-III. Blood 51:825-833, 1978.
10. TIMOTHY AR, SUTCLIFFE SBJ, WRIGLEY PFM, ET AL. Hodgkin's disease: combination chemotherapy for relapse following radical radiotherapy. Int J Radiat Oncol Biol Phys 5:165-169, 1979.
11. DEVITA VT, SIMON RM, HUBBARD SM, ET AL. Curability of advanced Hodgkin's disease with chemotherapy. Ann Intern Med 92:587-595, 1980.
12. MAUCH P, RYBACK ME, ROSENTHAL D, ET AL. The influence of initial pathologic stage on the survival of patients who relapse from Hodgkin's disease. Blood 56:892-897, 1980.
13. CADMAN E, BLOOM AF, PROSNITZ L, ET AL. The effective use of combined modality therapy for the treatment of patients with Hodgkin's disease who relapsed following radiotherapy. Am J Clin Oncol 6:313-318, 1983.
14. BONADONNA G, MONFARDINI S, DE LENA M, ET AL. Phase I and preliminary phase II evaluation of adriamycin (NSC-123127). Cancer Res 30:2572-2582, 1970.
15. BONADONNA G, DE LENA M, MONFARDINI S, ET AL. Clinical trials with bleomycin in lymphomas and in solid tumors. Eur J Cancer 8:205-215, 1972.
16. DE LENA M, MONFARDINI S, BERETTA G, ET AL. Clinical trials with intensive chemotherapy and radiotherapy in Hodgkin's disease. Natl Cancer Inst Monogr 36:403-420, 1973.
17. BONADONNA G, ZUCALI R, MONFARDINI S, ET AL. Combination chemotherapy of Hodgkin's disease with adriamycin, bleomycin, vinblastine, and imidazole carboxamide versus MOPP. Cancer 36:252-259, 1975.
18. BONADONNA G, and SANTORO A. ABVD chemotherapy in the treatment of Hodgkin's disease. Cancer Treat Rev 9:21-35, 1982.
19. BONADONNA G. Chemotherapy strategies to improve the control of Hodgkin's disease: the Richard and Hilda Rosenthal Foundation Award Lecture. Cancer Res 42:4309-4320, 1982.
20. SANTORO A, VIVIANI S, ZUCALI R, ET AL. Comparative results and toxicity of MOPP versus ABVD combined with radiotherapy in PS IIB, III (A, B) Hodgkin's disease. Proc ASCO 4:199, 1985.
21. SANTORO A, BONADONNA G, BONFANTE V, ET AL. Alternating drug combinations in the treatment of advanced Hodgkin's disease. N Engl J Med 306:770-775, 1982.
22. BONADONNA G, SANTORO A, VALAGUSSA P, ET AL. Current status of the Milan trials for Hodgkin's disease in adults. *In* Proceedings of the Second International Conference on Malignant Lymphomas (Rozencweig M, Cavalli F, Bonadonna G, eds). Boston, Martinus Nijhoff Publishing, 1985. In press.
23. BONADONNA G, VALAGUSSA P, and SANTORO A. Prognosis of bulky Hodgkin's disease treated with chemotherapy alone or combined with irradiation. Cancer Survey, 1985. In press.
24. PETO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 35:1-39, 1977.
25. BERGSAGEL DE, ALISON RE, BEAN HA, ET AL. Results of treating Hodgkin's disease without a policy of laparotomy staging. Cancer Treat Rep 66:717-731, 1982.
26. HAYBITTLE JL, EASTERLING MJ, BENNETT MH, ET AL. Review of British National Lymphoma Investigation studies of Hodgkin's disease and development of prognostic index. Lancet i:967-972, 1985.
27. GOMEZ GA, REESE PA, NAVA H, ET AL. Staging laparotomy and splenectomy in early Hodgkin's disease. Am J Med 77:205-210, 1984.
28. TUBIANA M, HENRY-AMAR M, HAYAT M, ET AL. The EORTC treatment of early stages of Hodgkin's disease: the role of radiotherapy. Int J Radiat Oncol Biol Phys 10:197-210, 1984.
29. COOPER MR, PAJAK TF, GOTTLIEB AJ, ET AL. The effects of prior radiation therapy and age on the frequency and duration of complete remission among various four-drug treatments for advanced Hodgkin's disease. J Clin Oncol 2:748-755, 1984.
30. BAKEMEIER RF, ANDERSON JR, COSTELLO W, ET AL. BCVPP chemotherapy for advanced Hodgkin's disease: evidence for greater duration of complete remission, greater survival and less toxicity than with a MOPP regimen. Ann Intern Med 101:447-456, 1984.
31. SUTCLIFFE SB, WRIGLEY PFM, and PETO J. MVPP chemotherapy regimen for advanced Hodgkin's disease. Br Med J 1:679-683, 1978.
32. O'BRYAN RM, LUCE JK, TALLEY RW, ET AL. Phase II evaluation of adriamycin in human neoplasia. Cancer 32:1-8, 1973.
33. BONADONNA G, BERETTA G, TANCINI G, ET AL. Adriamycin (NSC-123127) studies at the Istituto Nazionale Tumori, Milan. Cancer Chemother Rep Part 3, vol 6(2):231-245, 1975.
34. VALAGUSSA P, SANTORO A, FOSSATI-BELLANI F, ET AL. Absence of treatment induced second neoplasms after ABVD in Hodgkin's disease. Blood 59:488-494, 1982.
35. VALAGUSSA P, SANTORO A, FOSSATI-BELLANI F, ET AL. Second neoplasms in Hodgkin's disease: progress report. Proc Am Assoc Cancer Res 26:182, 1985.
36. VIVIANI S, SANTORO A, RAGNI G, ET AL. Gonadal toxicity after combination chemotherapy for Hodgkin's disease. Comparative results of MOPP versus ABVD. Eur J Cancer Clin Oncol 21:601-605, 1985.

Electrocardiographic Monitoring of Patients Receiving Phase I Cancer Chemotherapy[1,2]

Marla R. Hersh, William Linn, John G. Kuhn,* and Daniel D. Von Hoff[3,4]

Previous reports have characterized the various non-drug-induced cardiac disorders in cancer patients as well as the cardiotoxicity of commonly used anticancer agents. This study was conducted to determine the non-drug-related incidence of arrhythmias in patients receiving phase I antineoplastics. Twenty-four-hour Holter monitors were placed on 36 consecutive patients about to receive phase I agents. A 1–4-hour baseline was obtained before treatment was begun. Sixty-four percent of the patients observed had a wide variety of ventricular and supraventricular dysrhythmias which did not increase during or after chemotherapy. Eighty-four percent of these arrhythmias were not detected during routine monitoring consisting of baseline 12-lead ECGs and 1-minute rhythm strips prior to, during, and after treatment, but were detected with the Holter monitor. Thirty-six percent of the dysrhythmias detected by the Holter monitor were considered potentially treatable arrhythmias requiring further evaluation and possible therapeutic intervention. During the early development of phase I agents, careful evaluation should be exercised so as not to confuse pre-existing, undiagnosed arrhythmias with drug-induced changes. To more accurately determine the true arrhythmogenic potential of phase I agents, Holter monitoring should be considered as a standard part of patient assessment prior to entry of a patient in phase I trials. [Cancer Treat Rep 70:349–352, 1986]

Recent reviews have characterized the various non-drug-induced cardiac disorders in cancer patients as well as the cardiotoxicity of the commonly employed anticancer agents (1–3). It has been difficult to determine whether these dysrhythmias were due to the arrhythmogenic properties of the agents themselves, patient anxiety associated with receiving an investigational agent, or the patient's baseline rhythm.

In a double-blind cross-over study, Mayernik et al (4) placed Holter monitors on 20 patients receiving either placebo or phase I or II agents, including diaziquone, mitoxantrone, aclarubicin, pyran copolymer (1:2, copolymer of maleic anhydride and divinyl ether), pentostatin, and homoharringtonine. Seventeen of the 20 patients monitored had arrhythmias during baseline, control, or posttherapy recording. Only two of the 17 arrhythmias observed appeared to be drug-related, and they concluded that arrhythmias were not a major problem with the drugs they studied.

The present study was conducted to determine the incidence and type of arrhythmias in patients about to receive phase I antineoplastic agents.

MATERIALS AND METHODS

A two-channel Holter monitor (Electrocardiocorder, Model 445B) was placed on 36 consecutive patients receiving phase I therapy with one of four agents, including echinomycin, a bifunctional intercalator (20 patients); fludarabine, an antimetabolite (seven); tiazofurin, an antimetabolite (four); or menogaril, an anthracycline antibiotic (five). All patients who were entered in the study had histological evidence of malignancy and an estimated survival of > 12 weeks. All patients had adequate blood cell counts (wbc count > 4000/μl; platelet count > 100,000/μl); bilirubin < 2.0 mg/dl; SGOT < 1.5 × normal; and creatinine clearance > 35 ml/minute. Patients whose laboratory parameters did not fall within

[1]Received Jan 30, 1985; revised June 24, 1985; accepted June 27, 1985.

[2]Supported in part by contract N01-CM-27542 (Division of Cancer Treatment) from the National Cancer Institute and Public Health Service grant RR-01346 (Division of Research Resources) from the National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medicine, Division of Oncology-Hematology (*M. R. Hersh, J. G. Kuhn,* and *D. D. Von Hoff*), and Pharmacology (*M. R. Hersh, W. Linn,* and *J. G. Kuhn*), University of Texas Health Science Center at San Antonio and the Audie L. Murphy Memorial Veterans Hospital, San Antonio.

[4]We thank the nursing and dietetic staff of the Audie L. Murphy Memorial Veterans Hospital Special Diagnostic and Treatment Unit and Mary Adams for her assistance in preparing this manuscript.

Reprint requests to: John G. Kuhn, MD, Department of Medicine, University of Texas Health Science Center, San Antonio, 7703 Floyd Curl Dr, San Antonio TX 78284.

these ranges could be entered if the abnormalities were due to their tumors. Patient characteristics and drug dosages are shown in table 1. Patients 25 and 32 had a prior history of hypertension, but the others had a negative cardiac history, including no previous history of angina, congestive heart failure, or myocardial infarction. No cardiac function studies other than an ECG are routinely done.

Electrocardiograms

Eligibility criteria for entry in the phase I clinical trial included a "normal cardiac status" as indicated by a 12-lead ECG.

Rhythm Strips

Patient cardiac status was monitored during treatment with 1-minute rhythm strips at regularly scheduled times before, during, and for 24-48 hours after infusion. Rhythm strips were obtained every 15 minutes for the first hour of the infusion, then every hour for 2 hours, and then every 6 hours for the remainder of the study period.

Holter Monitoring

A 1-4-hour (2-hour, 15-minute average) baseline Holter monitoring was done before initiation of chemotherapy and patients were continuously monitored during and after therapy, for a total of 24 hours. Obtaining baseline recordings allowed each patient to serve as his or her own control. The Holter tapes were read by a trained observer and the arrhythmias recorded.

With this study design, we could compare readings of 12-lead ECG, rhythm strips, and Holter monitors as baselines for administration of phase I agents.

RESULTS

Of the 36 patients (seven females, 29 males) monitored, only two patients (Nos. 20 and 25) had baseline 12-lead

TABLE 1.—Patient characteristics

Patient No.	Drug*	Dose		Age (yrs)	Disease
		mg/m^2	mg		
1	E	60	118.8	60	Colon
2	E	60	94.8	59	Head and neck
3	E	120	211.0	72	Lung (squamous)
4	E	120	207	65	Liver
5	E	120	228	64	Renal
6	E	180	300	66	Lung (squamous)
7	E	241	421	55	Unknown primary
8	E	300	588	32	Colon
9	E	180	369	75	Colon
10	E	420	789	44	Renal
11	E	420	693	60	Colon
12	E	540	1284	47	Renal
13	E	540	840	70	Colon
14	E	540	777	51	Lung (large cell)
15	E	660	1089	63	Head and neck
16	E	660	1042.8	70	Lung (squamous)
17	E	660	1167	44	Colon
18	E	780	1230	60	Fallopian
19	E	1200	1840	61	Head and neck
20	E	2128	3873	67	Colon
21	F	18	31	57	Colon
22	F	25	51	57	Lung (large cell)
23	F	18	27	51	Lung (large cell)
24	F	25	48	32	Colon
25	F	18	32	56	Colon
26	F	18	29	56	Hepatoma
27	F	18	28	41	Breast
28	T	550	1056	75	Colon
29	T	550	946	48	Cholangiocarcinoma
30	T	550	847	60	Lung (squamous)
31	T	1100	1914	64	Renal
32	M	16	24	57	Colon
33	M	16	30	33	Colon
34	M	32	53	51	Lung (squamous)
35	M	52	100	53	Lung (large cell)
36	M	52	81	74	Head and neck

* E = echinomycin; F = fludarabine; T = tiazofurin; and M = menogaril.

TABLE 2.—Cardiac recordings*

Patient No.	12-lead ECG	Rhythm strip	Holter†
1	Nonspecific T-wave change	NC	N
2	NSR	NC	N
3	RBBB, PAC	NC	200–300 PAC
4	WNL	NC	10–15 PAC
5	LAD, LAFB	Rare PAC/NC	N
6	WNL	NC	N
7	WNL	NC	N
8	WNL	NC	STΔ-position
9	Supra PVC	Frequent PAC	2000–3000 PAC; frequent supraventricular couplet
10	ST	Rare PVC, ST	Rare PAC, MFPVC
11	WNL	NC	N
12	NSR	NC	0–60 MFPVC
13	WNL	Frequent PAC, PVC/NC	Frequent PVC, PAC
14	1° AV block	NC	Acc./nodal, EAT
15	WNL	—	Rare MFPVC
16	NSR	NC	5-beat EAT
17	WNL	NC	Tachybrady†, 2-sec pause
18	—	—	Frequent PAC; 80–100 MFPVC; occasional fusion beat
19	NSR	—	Frequent atrial; bigeminy; rare UPVC
20	Irregular rate; flip T waves; ? sick sinus syndrome	—	Rare UPVC; 4:3 Wenckebach
21	NSR, RBBB, LAD	Occasional PAC	Rare UPVC
22	Left atrial abnormal	NC	N
23	WNL	NC	N
24	WNL	NC	N
25	Frequent PVCs; Q waves in lead 3; left ventricular hypertrophy	Occasional PVC	10–20 MFPVC, 1 couplet
26	Nonspecific STΔ; poor R-wave progression	Rare PVC/NC	N
27	WNL, ST	—	N
28	PACs STΔ	Frequent PAC/NC	600–1800 PAC; occasional supraventricular couplet
29	WNL	NC	Rare MFPVC
30	Sinus arrhythmia; LVH	Frequent PVC; occasional PAC, 1 couplet	20–30 MFPVC
31	WNL, LAD	Occasional PAC/NC	10–20 PVC
32	NSR	Occasional PAC/NC	Rare PAC
33	WNL	NC	N
34	RAD	NC	N
35	NSR	NC	Rare PAC, ST, STD
36	NSR	NC	Occasional PVC; rare MFPVC; rare PAC

*NSR = normal sinus rhythm; RBBB = right bundle branch block; PAC = premature atrial contractions; WNL = within normal limits; LAD = left axis deviation; LAFB = left anterior fascicular block; Supra PVC = supraventricular PVC; ST = sinus tachycardia; NSR = normal sinus rhythm; 1° AV block = 1st degree atrioventricular block; STΔ = ST-T-wave changes; LVH = left ventricular hypertrophy; RAD = right axis deviation; NC = no change; MFPVC = multiformed PVC; Acc./nodal = accelerated nodal rhythm; EAT = ectopic atrial tachycardia; UPVC = uniform PVC; and STD = ST-wave depression.

†No. of PACs and PVCs/hr.

‡Tachybrady = tachybrady syndrome. Term used to describe tachyarrhythmia components occurring intermittently during a slow rhythm; this phenomenon has been associated with SSS.

ECGs which revealed potential abnormalities (table 2). Only Patient 30 had serious arrhythmias noted on his 1-minute rhythm strips (table 2).

The arrhythmias noted during Holter monitoring occurred during baseline monitoring as well as during and after treatment, indicating that none of the 36 patients exhibited evidence of drug-induced dysrhythmias. Two patients (Nos. 8 and 35) had nonspecific ST-T wave changes which could have been position-related. Thirteen patients (36%) had negative Holter monitors. The remainder of the patients (64%) had a wide variety of supraventricular and ventricular dysrhythmias (table 3).

Patients 17 and 25 (5%) had malignant ectopy. Six patients (Nos. 10, 11, 15, 16, 18, and 30) (17%) had multiformed premature ventricular contractions (PVCs). Another five patients (Nos. 9, 14, 19, 20, and 28) (14%) had potentially treatable arrhythmias. Thus, 13 of 36 patients (36%) observed had potentially treatable arrhythmias. The frequency of ectopy did not increase during or after drug therapy. One patient developed short runs of supraventricular tachycardia during episodes of nausea and vomiting.

TABLE 3.—Types of cardiac dysrhythmias

Supraventricular	Ventricular
Frequent PACs	Frequent PVCs (uniformed and multiformed)
Sinus exit block	Fusion beats
EAT	Couplets
Sinus arrest	
2° AV block	

DISCUSSION

This study indicates that a high number of patients (64%) receiving phase I chemotherapy have arrhythmias. Thirty-six percent of the patients receiving chemotherapy (13) have potentially treatable arrhythmias. Patients having malignant ectopy would require further cardiac evaluations and treatment, while those having multiformed PVCs would have been considered for further cardiac evaluations (5). Both tachybrady syndrome or SSS and frequent and/or complex ventricular ectopy are associated with a high mortality rate, especially in the elderly (6–8). The five patients with potentially treatable arrhythmias would be treated if they were symptomatic. Only three (23%) of these arrhythmias were noted on the patients' baseline 12-lead ECGs or on the rhythm strips recorded prior to, during, and after treatment. Thus, 77% of the potentially treatable arrhythmias were noted exclusively on the Holter monitors.

The high incidence of arrhythmias observed in these patients also indicates that arrhythmias noted to occur during treatment cannot always be attributed to the antineoplastic agents themselves, but may be due to the patient's intrinsic rhythm. This same phenomenon was also noted by Mayernik et al (4). Most of the patients could continue their chemotherapy while being evaluated and treated for their arrhythmias, since there was no increase in the incidence of arrhythmias noted during and after administration of chemotherapy.

Unfortunately, this is a nonrandomized, nonblinded analysis in which only a mean 2-hour, 15-minute baseline Holter monitoring was done immediately prior to treatment with phase I antineoplastics. The short baseline monitoring period did not allow for normal variations due to changes occurring with sleep, exercise, meals, or normal diurnal alterations. The possibility of elevated catecholamines due to anxiety surrounding administration of phase I chemotherapy cannot be ruled out as a contributing factor for the arrhythmias. In addition, the population chosen may be at high risk for coronary artery disease due to their age (median, 60 years old) and possible smoking history (36% with lung or head and neck cancer). Other institutions may have younger patient populations and a different distribution of tumor types in their patients receiving phase I antineoplastics.

Based on the high incidence of serious cardiac arrhythmias observed in these patients receiving phase I agents and the failure of these arrhythmias to be noted during routine monitoring (12-lead ECGs and rhythm strips), patients receiving phase I treatment should be Holter-monitored prior to therapy to establish a baseline rhythm and identify potentially treatable arrhythmias. Perhaps the method for determining the baseline cardiovascular function in patients receiving phase I antineoplastics should be re-evaluated to include the use of Holter monitoring. Although these potentially treatable arrhythmias were discovered while preparing patients for treatment with these phase I agents, the impact on patient survival cannot be known because of their malignancies.

REFERENCES

1. VON HOFF DD, ROZENCWEIG M, and PICCART M. The cardiotoxicity of anticancer agents. Semin Oncol 9:23-33, 1982.
2. ROZENCWEIG M, PICCART M, and VON HOFF DD. Cardiac disorders in cancer patients. *In* Medical Complications in Cancer Patients (Klastersky J, and Staquet MJ, eds). New York, Raven Press, 1981, pp 211-229.
3. TOKAZ LK, and VON HOFF DD. The cardiotoxicity of anticancer agents. *In* Toxicity of Chemotherapy (Perry MC, and Yarbro JW, eds). New York, Grune and Stratton, 1984, pp 199-226.
4. MAYERNIK D, STEVENSON J, and NEIDHART J. Acute cardiac arrhythmias with phase I and II agents. Proc ASCO 18:14, 1982.
5. MORGANROTH J. Premature ventricular complexes, diagnosis and indications for therapy. JAMA 252:673-676, 1984.
6. AROESTY J, COHEN S, and MORKIN E. Bradycardia-tachycardia syndrome: results in twenty-eight patients treated by combined pharmacologic therapy and pacemaker implantation. Chest 66:257-263, 1974.
7. WOHL A, LAKORDE J, ATKINS J, ET AL. Prognosis of patients permanently paced for sick sinus syndrome. Arch Intern Med 136:406-408, 1976.
8. WINKLE R. Ambulatory electrocardiography and the diagnosis, evaluation and treatment of chronic ventricular arrhythmias. Prog Cardiovasc Dis 23:99-128, 1980.

Phase I–II Evaluation of Intra-arterial Diaziquone for Recurrent Malignant Astrocytomas[1,2]

Harry S. Greenberg,[3,*] William D. Ensminger,[3] Patricia B. Layton,[3] Stephen Gebarski,[3] Mark Meyer,[3] Bruce Chaffee,[3] John F. Bender,[4] and Antonio J. Grillo-Lopez[3,4]

Diaziquone (AZQ) is a lypophilic alkylating agent that crosses the blood-brain barrier and has shown broad activity in animal tumor models. Five of 12 patients with malignant astrocytoma treated with iv AZQ had clinical and/or radiographic improvement (Schold, Neurology 34:615, 1984). Intra-arterial administration of AZQ to patients with brain tumors should produce higher peak levels of drug in the tumor and should reduce systemic toxicity. Twenty-one patients with astrocytoma (grade II, four; grade III, 11; and grade IV, six), in all of whom irradiation and intra-arterial carmustine chemotherapy failed, received intra-arterial AZQ as a single dose every 28 days. Two of 20 evaluable patients experienced partial responses of 5 and 8+ months, respectively. Four patients had disease stabilization of 3, 4, 5, and 8 months' duration, respectively, and one of these patients had tumor shrinkage (partial response) after seven courses of AZQ. The initial dose in the first three patients was 10 mg/m^2, and doses in subsequent groups of three patients were begun at increases of 5 mg/m^2. The within-group dose escalation was 5 mg/m^2 per course if there was no hematologic toxicity. Dose-limiting toxicity was myelosuppression, which occurred at doses > 15 mg/m^2. The maximum tolerated dose was 25 mg/m^2. Intra-arterial AZQ appears to be of marginal effectiveness in patients refractory to carmustine and offers no advantage over iv AZQ in efficacy or toxicity. [Cancer Treat Rep 70:353–357, 1986]

Malignant high-grade astrocytomas are uniformly and rapidly fatal, even with appropriate medical treatment (1–6). Data from the initial comparative trial conducted by the Brain Tumor Study Group indicated that median survival in postsurgical patients was extended from 17 weeks to 37 weeks with radiotherapy (2). Single-agent use of the nitrosoureas following surgery has provided only marginal benefit (2, 5, 7). Results with the combination of radiation therapy and carmustine (BCNU) have extended the median survival to only 51 weeks (5).

Diaziquone (AZQ) is an aziridinylbenzoquinone of low molecular weight and high lipid solubility, and it is not ionized at physiologic pH. It attains rapid and high concentrations in the central nervous system following iv administration. Although the exact mechanism of action of AZQ is not known, it is presumed to be an alkylating agent. In phase II trials in patients with malignant high-grade astrocytomas who had failed BCNU, AZQ produced a response rate of between 15% and 20% when administered iv (8).

Preclinical data and computer modeling information indicate that an alkylating agent such as AZQ with its short half-life would be an ideal candidate for intra-arterial administration, allowing a higher peak tumor concentration of drug and potentially reducing systemic toxicity (*see* Discussion section). To evaluate this premise, we initiated a phase I–II clinical trial of intra-arterial AZQ in patients with refractory malignant astrocytomas who had failed radiation therapy and intra-arterial BCNU therapy.

MATERIALS AND METHODS

Twenty-one consecutive patients with unilateral histologically proven malignant astrocytoma were entered. All patients had to have (*a*) failed surgical therapy; (*b*) failed external beam radiotherapy; (*c*) failed intra-arterial BCNU chemotherapy; (*d*) measurable disease with evidence of progression; (*e*) expected survival of > 4 weeks; (*f*) performance status (Zubrod) of 3 or better; (*g*) adequate time to recover from prior significant chemotherapeutic toxicity; and (*h*) an absolute granulocyte count of

[1]Received Feb 7, 1985; revised June 3, 1985; accepted June 13, 1985.
[2]Supported by a grant from Warner-Lambert/Parke-Davis.
[3]The University of Michigan Hospitals, Ann Arbor.
[4]Warner-Lambert/Parke-Davis, Pharmaceutical Research Division, Clinical Oncology Research Program, Ann Arbor, MI.
[*]*Reprint requests to:* Harry S. Greenberg, MD, Department of Neurology, B4913 CFOB, Box M056, University of Michigan Hospitals, Ann Arbor, MI 48109.

$\geq 1500/mm^3$ and a platelet count of $\geq 150,000/mm^3$. Patients were excluded from entry if they had: (*a*) received radiotherapy within 2 weeks or nitrosourea therapy within 6 weeks prior to treatment with AZQ; (*b*) impaired hepatic function (bilirubin > 1.5 mg/100 ml or SGOT > 60 IU/ml) or renal function (creatinine > 2.0 mg/100 ml); and (*c*) other neurologic illness (dementia, multiple sclerosis, amyotrophic lateral sclerosis, etc) that would obscure the evaluation of response. Patients with visual problems secondary to intra-arterial BCNU were not excluded. Patients were informed of the investigational nature of the study and gave written informed consent. AZQ was initially administered at a dose of 10 mg/m^2 in a concentration of 1 mg/ml to be infused intra-arterially at 5 mg/minute as a single dose every 28 days. Patients were brought into the study in sets of three patients per dose level. Each succeeding dose level was escalated by 5 mg/m^2 until dose-limiting toxicity occurred (absolute granulocyte count nadir $< 500/mm^3$ and platelet count nadir $< 50,000/mm^3$). Patients not achieving a granulocyte count nadir of between 1000 and $1500/mm^3$ received a 20% dosage increase (provided that the platelet count nadir was $> 75,000/mm^3$). Patients with granulocyte count nadirs $< 1000/mm^3$ or platelet count nadirs $< 75,000/mm^3$ received a 20% dosage reduction. Subsequent courses were delayed until full hematologic recovery (granulocyte count $> 1500/mm^3$, platelet count $> 100,000/mm^3$) had occurred.

During therapy, patients were evaluated with: medical history, physical and neurologic examination, performance status, chest x-ray, ECG, computerized axial tomographic (CAT) scan, chemical profile (glucose, BUN, total protein, albumin, calcium, SGOT, LDH, alkaline phosphatase, uric acid, and bilirubin), serum creatinine, serum electrolytes, cbc, platelets, and urinalysis. Cbc and platelet counts were obtained weekly. Response criteria were as follows: *complete response*—complete resolution of all abnormalities on CAT scan and an improved or normal neurologic examination without steroid medication; *partial response*—a decrease in the size of the measurable mass lesion on CAT scan plus a stable or improved neurologic examination on stable or decreasing doses of steroids; *stable disease*—no change in the size of the tumor on CAT scan without significant change in the neurologic examination on stable or decreasing doses of steroids; and *progressive disease*—increase in the size of the tumor on CAT scan or progressive worsening of neurologic signs and symptoms directly attributable to the growth and effects of the tumor. The duration of response dated from the onset of response to the documentation of disease progression.

Drug Delivery System

Patients were treated as described previously by Greenberg et al (9) with AZQ administered through a selective internal carotid (18 patients) or vertebral artery catheter (two patients) placed percutaneously using the femoral approach or through the sideport of an Infusaid permanently implantable intracarotid drug delivery system (manufactured by Infusaid Corp, Norwood, MA) (one patient). In all internal carotid artery infusions, the catheter was below the ophthalmic artery.

AZQ (supplied by Warner-Lambert/Parke-Davis) was reconstituted immediately prior to administration utilizing the standard tripack formulation developed by the National Cancer Institute. A 10-ml vial containing 10 mg of lyophilized AZQ was reconstituted using 0.5 ml of the solvent *N,N*-dimethylacetamide (DMA). Once the drug was completely dissolved in the DMA, 9.5 ml of phosphate buffer (0.01 M, pH 6.5) was added, resulting in a solution containing 1 mg/ml of AZQ with 5% vol/vol DMA in pH 6.5 phosphate buffer. The drug was then infused at a concentration of 1 mg/ml intra-arterially at 5 mg/minute as a single dose using an IMED 922 (Medical Electronics Corp) infusion pump every 28 days. During the infusion, pulse and blood pressure were monitored at regular intervals.

RESULTS

Twenty-one patients with primary malignant tumors of the central nervous system were entered on study between June 1982 and May 1984 at the University of Michigan Hospital. Patient characteristics are shown in table 1. One patient was considered inevaluable due to a lack of evidence of progressive disease on entry. Of the remaining 20 patients, 16 had histologically proven grade III-IV astrocytoma and four had grade II astrocytoma. A total of 66 courses of intra-arterial AZQ were administered to these 21 patients. Dosage escalations proceeded from 10 to 30 mg/m^2 (table 2). The dose-limiting toxicity in this study was bone marrow suppression (tables 2 and 3). Two of 20 evaluable patients (10%) experienced partial responses of 5 and 8+ months, respectively. Four patients had stable disease for 3, 4, 5, and 8 months, respectively (table 4). The latter patient, with a grade III astrocytoma, then had a partial response with decrease in tumor size for 4 additional months. Four patients experienced toxic effects other than bone marrow suppression. One patient developed focal left hand seizures approximately 10 minutes after intra-arterial infusion of the first course of AZQ. This progressed to status epilepticus, but 10 days later the patient had reverted to baseline except for a slight increase in aphasia. This patient had experienced seizures following prior treatment with intra-arterial BCNU. A second patient experienced increasing right hemiparesis, increasing headache, and nonfluent aphasia beginning approximately 1 hour after the first course of AZQ. This patient was treated with steroids and was discharged 36 hours later with a neurologic examination approaching baseline. This patient had also experienced these symptoms following prior treatment with intra-arterial BCNU. A third patient devel-

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	21
Sex	
Males	10
Females	11
Mean age in yrs (range): 47 (21-68)	
Mean performance status (range): 1 (0-3)	
Primary tumor (astrocytoma)	
Grade II	4
Grade III	11
Grade IV	6

oped severe retro-orbital right eye pain during intra-arterial infusion of AZQ lasting several days and requiring frequent narcotic analgesics. The right eyelid was swollen and the patient was unable to open the eye due to soft tissue swelling. Visual acuity in the right eye was decreased, although examination by ophthalmoscope did not show papilledema or retinal damage as seen in BCNU-induced retinal vasculitis. Twenty-six days after the incident, the patient still had some mild residual eye pain, photophobia, diplopia, and decreased visual acuity. A fourth patient had painless visual loss ipsilateral to the side of arterial infusion 2 weeks after the second course of AZQ at a dose of 25 mg/m^2. Vision continued to deteriorate and optic atrophy subsequently developed. No fundal hemorrhages or exudates were observed. Neither of the patients treated with vertebral artery AZQ had neurologic or autonomic complications.

DISCUSSION

Based on theoretical and experimental considerations, intra-arterial administration of a cytotoxic alkylating agent with a high lipid partition coefficient and a short biologic half-life should produce very high intratumor concentrations of drug and might possibly protect the host against toxic effects encountered with iv administration (10-12). Eckman et al (10) have assessed the theoretical advantage of intra-arterial drug administration. The relative advantage (R) of intra-arterial administration is defined as the ratio of tumor exposure following intra-arterial administration to that following iv administration, where exposure is the concentration-time integral. With linear kinetics of drug distribution and metabolism, it can be shown that R is independent of the rate of drug administration. Assuming clearance by the lungs is negligible, Eckman et al (10) have shown that R = 1 + CL/F, where F is flow in the artery infused (L/min) and CL is systemic drug clearance (L/min). Assuming equal partitioning of AZQ between plasma and rbcs, a carotid blood flow of 250 ml/minute, a body weight of 70 kg, and an AZQ plasma clearance of 11.2 ml/minute/kg (13), R is calculated to have a value of 3.1. The Infusaid pump system with increase in internal carotid artery blood flow secondary to ligation of the external carotid might have the potential to decrease a drug's regional advantage.

In dogs, Feun et al (14) and Egorin et al (15) have measured R of intracarotid 14C-AZQ by 30-minute and 4-hour infusions, respectively. Feun et al found R (the ratio of brain tissue of AZQ concentration of intra-arterial to iv administration) to be 2.8 at 1 hour, which fell to 1.8 in animals killed at 48 hours. Egorin et al found this ratio to be from 1.0 to 1.7 in three animals killed at 4 hours. Egorin et al concluded that intracarotid AZQ is unlikely to be of significant benefit, while Feun et al reached the opposite conclusion. The dog is not the ideal model for relative advantage measurements that will be applied to humans. A much higher percentage of flow is through the external rather than the internal carotid circulation, and with proximal occlusion of the common carotid a significant proportion of flow to the internal carotid artery distribution will be through collateral flow.

The calculated R for BCNU in a previous study was 5.7, which would suggest a greater relative advantage for BCNU. The exposure to tissues not directly perfused by the infused artery, including the contralateral hemisphere and bone marrow, is slightly less than that with an identical dose given iv. Studies by Rosenblum et al (11) suggest that for BCNU, AZQ, and alkylating agents in general, high peak concentrations are associated with increased tumoricidal effect. AZQ is such a compound. In this study, however, the efficacy of intra-arterial AZQ was less than that seen in most of the phase II trials (8, 16-19) of iv administered AZQ (eg, 15%-20%). One possible explanation is that in our study, 100% of the patients had failed intra-arterial BCNU, while in the iv

TABLE 2.—Myelosuppression by AZQ at each dose level

Dose (mg/m^2)	No. of patients	No. of patients without prior AZQ	No. of courses	Mean nadir count (range) × 10 cells/μl	
				Granulocytes	Platelets
10	3	3	3	2.9 (1.3-4.2)	No suppression
15	9*	3	16	2.6 (0.9-6.5)	132 (62-254)
20	10*	3	13	1.5 (0.1-2.7)	117 (18-218)
25	13*	10	25	2.0 (0.2-5.8)	142 (17-321)
30	5	2	9	1.9 (0-3.75)	80 (4-186)

* Includes patients with dose de-escalations.

TABLE 3.—Myelosuppression by AZQ with first dose

Dose (mg/m^2)	No. of patients	Mean nadir count (range) × 10 cells/μl	
		Granulocytes	Platelets
10	3	2.2 (1.3-3.2)	No suppression
15	3	3.9 (2.5-5.2)	190 (128-254)
20	3	1.6 (0.1-2.7)	80 (18-148)
25	10	2.6 (0.5-5.8)	165 (86-231)
30	2	0.75 (0-1.5)	44 (4-83)

TABLE 4.—Response by dose level and tumor grade

Dose (mg/m^2)	No. of patients	Tumor grade* —		
		II (n = 4)	III (n = 11)	IV (n = 6)
10	3	0	0	0
15	9	0	0	1 (PR)
20	10	0	1 (SD)†	0
25	13	1 (SD)	0	2 (PR, SD)
30	5	0	0	1 (SD)

*Values = No. of patients; SD = stable disease.
†Following SD, patient had a PR.

study of AZQ by Schold et al (19) with the highest response rate (43.8%), only 28% of the patients had received previous chemotherapy. The intra-arterial BCNU may have selected out clones of cells that would have been sensitive to intra-arterial AZQ, since both AZQ and BCNU are alkylating agents and those that have repopulated are now resistant to AZQ (20). The activity of intra-arterial AZQ may be greater in patients virgin to alkylating agents.

A second reason for the interest in intra-arterial AZQ for the treatment of primary brain tumors was because of the toxic effects experienced with intra-arterial BCNU in this patient population. If a new agent were as active as BCNU without producing similar toxic effects, this would be a definite advantage to patients. It is interesting to note that the two patients in this study who experienced seizures and increasing hemiparesis, respectively, following their first course of treatment with AZQ had experienced nearly identical neurologic signs when treated with intra-arterial BCNU. Seizures have also been reported as a complication of intra-arterial administration of cisplatin in patients with recurrent glioma (21).

The principal complication of intra-arterial BCNU chemotherapy has been toxic retinal vasculitis (22-25). Increased orbital vascularity and vasodilatation have been noted on angiography performed immediately after BCNU infusion, indicating a redistribution of flow to the retina (26). In this study, one patient experienced severe retro-orbital eye pain during the infusion of AZQ, but with minimal decrement in visual acuity. A second patient experienced a painless decrement in visual acuity beginning 2 weeks after the second course of AZQ, which continued to deteriorate to almost total blindness in the affected eye. The time course of this second incident is similar to that seen with BCNU vasculitis. Supraophthalmic intra-arterial infusion techniques have recently been reported which should help to eliminate this complication (27).

CONCLUSION

Intra-arterial administration of AZQ has limited efficacy in patients with primary malignant astrocytomas showing disease progression after proving refractory to radiotherapy and to nitrosourea therapy. In our study, we obtained a response rate of 9.5% (two PRs in 21 patients). With this response rate, the chance that AZQ has an actual response rate of ≥ 30% in this group of patients is 5%. The total single dose that can be safely administered intra-arterially to patients is approximately 25 mg/m^2. This is equivalent to the total dose of AZQ (27.5 mg/m^2) which can be administered iv over 5 days in terms of bone marrow suppression.

The activity of intra-arterial AZQ in patients previously untreated with chemotherapy is unknown.

REFERENCES

1. SHAPIRO WR, and YOUNG DF. Treatment of malignant glioma. A controlled study of chemotherapy and irradiation. Arch Neurol 33:494-500, 1976.
2. WALKER MD, ALEXANDER E, HUNT WE, ET AL. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. J Neurosurg 49:333-343, 1978.
3. LEVIN VA, HOFFMAN WF, PISCHER TL, ET AL. BCNU-5-fluorouracil combination therapy for recurrent malignant brain tumors. Cancer Treat Rep 62:2071-2076, 1978.
4. WILSON CB. Brain tumors. N Engl J Med 300:1469-1471, 1979.
5. WALKER MD, GREEN SB, BYAR DP, ET AL. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 303:1323-1329, 1980.
6. GREEN SB, BYAR DP, WALKER MD, ET AL. Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma. Cancer Treat Rep 67:121-132, 1983.
7. WILSON CB, GUTIN P, BOLDREY EB, ET AL. Single-agent chemotherapy of brain tumors. Arch Neurol 33:739-744, 1976.
8. BENDER JF, GRILLO-LOPEZ AJ, and POSADA JG. Diaziquone (AZQ). Invest New Drugs 1:71-84, 1983.
9. GREENBERG HS, ENSMINGER WD, CHANDLER WF, ET AL. Intra-arterial BCNU chemotherapy for treatment of malignant gliomas of the central nervous system. J Neurosurg 61:423-429, 1984.
10. ECKMAN WW, PATLAK CS, and FENSTERMACHER JD. A critical evaluation of the principles governing the advantages of intra-arterial infusions. J Pharmacokinet Biopharm 2:257-285, 1974.
11. ROSENBLUM ML, GEROSA MA, DOUGHERTY DV, ET AL. Improved treatment of a brain tumor model. I. Advantages of single- over multiple-dose BCNU schedules. J Neurosurg 58:177-182, 1983.
12. FENSTERMACHER JD, and COWLES AL. Theoretic limitations of intracarotid infusions in brain tumor chemotherapy. Cancer Treat Rep 61:519-526, 1977.
13. FRYTAK S, EAGAN RT, AMES MM, ET AL. Phase I study of diaziquone. Cancer Treat Rep 68:975-978, 1984.
14. FEUN LG, SAVARAJ N, LU K, ET AL. The pharmacologic fate of 2,5-diaziridinyl-3,6-bis(carboethoxyamino)1,4-benzoquinone (AZQ, NSC-182986) by intracarotid or intravenous administration in beagles. J Neurooncol 1:219-224, 1983.
15. EGORIN MJ, BELLIS EH, and SALCMAN M. The pharmacology of diaziquone given by intravenous or intracarotid infusion to normal

and intracranial tumor bearing puppies. J Neurosurg 60:1005–1013, 1984.
16. EAGAN RT, DINAPOLI RP, HERMANN RC, ET AL. Preliminary communication—treatment of primary brain tumors recurrent after irradiation with aziridinylbenzoquinone (AZQ; NSC-182986). Am J Clin Oncol 6:577–578, 1983.
17. CURT GA, KELLEY JA, KUFTA CV, ET AL. Phase II and pharmacokinetic study of aziridinylbenzoquinone [2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone, diaziquone, NSC-182986] in high grade gliomas. Cancer Res 43:6102–6105, 1983.
18. FEUN LG, YUNG WKA, LEAVENS ME, ET AL. A phase II trial of 2,5-diaziridinyl 3,6-bis(carboethoxyamino) 1,4-benzoquinone (AZQ, NSC-182986) in recurrent primary brain tumors. J Neurooncol 2:13–17, 1984.
19. SCHOLD SC, FRIEDMAN HS, BJORNSSON TD, ET AL. Treatment of patients with recurrent primary brain tumors with AZQ. Neurology 34:615–619, 1984.
20. YUNG WKA, SHAPIRO JR, and SHAPIRO WR. Heterogenous chemosensitivities of subpopulations of human glioma cells in culture. Cancer Res 42:992–998, 1982.
21. FEUN LG, WALLACE S, STEWART DJ, ET AL. Intracarotid infusion of *cis*-diamminedichloroplatinum in the treatment of recurrent malignant brain tumors. Cancer 54:794–799, 1984.
22. DEWYS WD, and FOWLER EH. Report of vasculitis and blindness after intracarotid injection of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) in dogs. Cancer Chemother Rep 57:33–40, 1973.
23. GREENBERG HS, ENSMINGER WD, SEEGER JF, ET AL. Intra-arterial BCNU chemotherapy for the treatment of malignant gliomas of the central nervous system: a preliminary report. Cancer Treat Rep 65:803–810, 1981.
24. KAPP J, VANCE R, PARKER JL, ET AL. Limitations of high dose intraarterial 1,3 bis(2-chloroethyl)-1-nitrosoures (BCNU) chemotherapy for malignant gliomas. Neurosurgery 10:715–719, 1982.
25. SHINGLETON BJ, BEINFANG DC, ALBERT DM, ET AL. Ocular toxicity associated with high-dose carmustine. Arch Ophthalmol 100:1766–1772, 1982.
26. GEBARSKI SS, GREENBERG HS, GABRIELSEN TO, ET AL. Orbital angiographic changes after intracarotid BCNU chemotherapy. J Neuroradiol 5:55–58, 1984.
27. KAPP JP, ROSS RL, and TUCKER EM. Supraophthalmic carotid infusion for brain tumor chemotherapy. Technical note. J Neurosurg 58:616–618, 1983.

Disposition of Tricyclic Nucleoside-5′-monophosphate in Blood and Plasma of Patients During Phase I and II Clinical Trials[1,2]

Garth Powis,* Peter J. Basseches, David M. Kroschel, Ronald L. Richardson, Michael J. O'Connell, and Larry K. Kvols[3,4]

Tricyclic nucleoside-5′-monophosphate (TCN-P) and its dephosphorylated metabolite tricyclic nucleoside (TCN) have been measured in the blood and plasma of patients receiving TCN-P by rapid iv infusion in a phase I trial at daily doses of 24–55 mg/m^2 for 5 days and in patients receiving TCN-P in a phase II trial at a single dose of 250 mg/m^2. TCN-P was rapidly accumulated by rbcs and had an initial half-life in blood of 6.1 hours and a terminal half-life of 89.2 hours. Total-body blood clearance of TCN-P was 2.6 ml/minute/m^2. The concentration of TCN-P in blood was not related to the dose of TCN-P and did not increase over 5 days' administration in the phase I patients. Plasma contained little detectable TCN-P even 5 minutes after administration. Plasma contained low concentrations of TCN, up to 0.4 μg/ml, which were maintained over several days. TCN did not accumulate in the plasma with repeated administration of TCN-P in the phase I patients. No other metabolites of TCN-P, apart from TCN, were detected in blood or plasma. No relationship was detected between pharmacokinetics and toxic response of TCN-P in the phase II patients. [Cancer Treat Rep 70:359–362, 1986]

Tricyclic nucleoside-5′-monophosphate (TCN-P) (fig 1) is an antitumor agent with activity in several animal tumor systems (1,2). TCN-P is an inhibitor of DNA, RNA, and protein synthesis and shows selective toxicity to cells in S phase (3). Unlike other nucleoside antitumor agents, TCN-P is not phosphorylated beyond the level of the monophosphate and is not incorporated into polynucleotides (4,5). TCN-P is dephosphorylated to tricyclic nucleoside (TCN) by an enzyme(s) present in plasma and by cellular ecto-5′-nucleotidase (6,7). TCN enters cells and can be rephosphorylated to TCN-P by adenosine kinase (5). The active form of the drug appears to be TCN-P (6). In this paper we report the pharmacokinetics of TCN-P and TCN in the blood and plasma of patients receiving TCN-P in phase I and II clinical trials.

PATIENTS AND METHODS

The disposition of TCN-P was studied in 12 patients receiving TCN-P by rapid iv infusion daily for 5 days at doses of 24, 36, 44, and 55 mg/m^2/day as part of a phase I clinical study. TCN-P disposition was also studied in six patients with advanced colorectal carcinoma receiving TCN-P at a single iv dose of 250 mg/m^2 as part of a phase II clinical study. All patients had a serum creatinine < 1.5 mg/dl, direct reacting bilirubin within normal values, and SGOT and alkaline phosphatase no more than two times the upper limit of normal. TCN-P was administered in a minimum of 50 ml of 5% dextrose in water over 10–15 minutes.

Blood (10 ml) was collected into chilled heparinized tubes containing 10 μg of adenosine-5′-monophosphate to inhibit possible dephosphorylation of TCN-P by plasma enzymes (8). In phase I patients, blood was collected at various times up to 6 hours on Day 1 of treatment and at 24-hour intervals thereafter for 4 days immediately before the patient received the next dose of TCN-P. In phase II patients, blood was collected at various times over 3 days after receiving TCN-P. The blood was divided into two fractions and plasma prepared from one of the fractions as soon as possible. Plasma and blood were stored frozen at -70°C until assay.

[1]Received Apr 1, 1985; revised May 14, 1985; accepted May 22, 1985.

[2]Supported by contract CM-27548 (Division of Cancer Treatment) and by Public Health Service grants CA-15083 and CA-31224 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Divisions of Developmental Oncology Research (*G. Powis, P. J. Basseches,* and *D. M. Kroschel*) and Medical Oncology (*R. L. Richardson, M. J. O'Connell,* and *L. K. Kvols*), Department of Oncology, Mayo Clinic and Foundation, Rochester, MN.

[4]We thank Dr. John Kovach for his helpful discussions.

**Reprint requests to:* Dr. Garth Powis, Division of Developmental Oncology Research, Department of Oncology, Mayo Clinic and Foundation, Rochester, MN 55905.

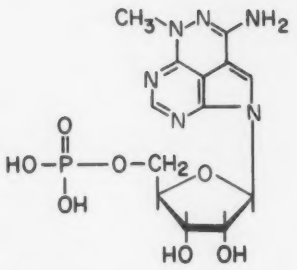

FIGURE 1.—Structure of TCN-P.

TCN-P and TCN were assayed by a modification of a reverse-phase high-performance liquid chromatographic assay described previously (7). Briefly, a 1-ml sample of blood or plasma was mixed with 4 ml of 0.5 N perchloric acid at 4°C, containing 10 μg of aminopyrine internal standard, and centrifuged at 1000 × *g* for 10 minutes to remove protein; the supernatant was applied to a 3-ml disposable RP-8 extraction cartridge (J. T. Baker, Phillipsburg, NJ). After washing the cartridge with water it was eluted with 4 × 2 ml of 90% methanol adjusted to pH 10.0 with ammonium hydroxide, and the eluate evaporated to dryness under a stream of N_2 at 30°C. The residue was dissolved in 50 μl of water and 25 μl taken for high-performance liquid chromatography on a 25-cm RP-8, 5-μm column (LiChrosorb, Merck, Darmstadt, West Germany) with a 5%–100% 10-minute linear gradient of methanol in 0.1 M sodium acetate, pH 5.3, at a flow of 1.5 ml/minute. The equipment used was a Hewlett-Packard 1090 high-performance liquid chromatograph with a diode array multiple wavelength detector. TCN was detected by its absorbance at 292 nm and, simultaneously, TCN-P was detected by its absorbance at 307 nm. The dual wavelength detection eliminated an endogenous peak in plasma and blood which interfered with detection of TCN-P at 292 nm, but which did not interfere at 307 nm. The limit of detection of the assay for TCN-P in plasma and blood was 50 ng/ml; for TCN in plasma, 25 ng/ml; and for TCN in blood, 100 ng/ml.

Blood and plasma drug concentration data were subjected to nonlinear least-squares regression analysis using the NONLIN computer program (9) and pharmacokinetic parameters calculated (10).

RESULTS

Phase I Studies

A typical blood and plasma concentration-time plot for TCN-P and TCN in a patient receiving TCN-P at 44 mg/m² as part of the phase I study is shown in figure 2. In all patients, TCN-P was rapidly removed from the plasma and even 5 minutes after administration, concentrations of TCN-P in the plasma were below the limit of detection

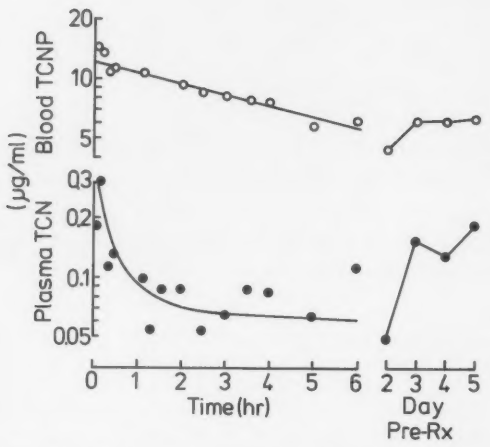

FIGURE 2.—Whole blood TCN-P (○) and plasma TCN (●) concentrations in a patient receiving TCN-P (44 mg/m²). No TCN-P could be detected in plasma. Concentrations are also shown on the 4 following days of treatment just before administering the next dose of TCN-P.

of the assay (50 ng/ml). TCN-P achieved high concentrations in whole blood at a maximum of 5 minutes after administration of the drug. TCN was detected in plasma at relatively low, but maintained concentrations. TCN could not be detected in samples of whole blood because of the lower sensitivity of the assay for TCN in blood. Pharmacokinetic parameters for TCN-P in blood and for TCN in plasma of 12 patients from the phase I study are shown in table 1. An initial half-life of TCN-P in the blood measured over 6 hours is given, but there were insufficient data to characterize the terminal phase of TCN-P elimination between 6 and 24 hours. There was no apparent relationship between the dose of TCN-P administered and the concentration of TCN-P in blood. TCN-P in blood and TCN in plasma did not show significant accumulation over the 5 days of treatment.

Phase II Studies

Because a slower second phase of TCN-P elimination from blood could be detected but not characterized in the phase I pharmacokinetic studies, six more patients receiving TCN-P at a dose of 250 mg/m² in a phase II trial were studied over 3 days. Plasma and blood TCN-P and TCN concentration data for these patients are shown in figure 3. Pharmacokinetic parameters for TCN-P in blood and TCN in plasma of individual patients are shown in table 2. In all patients, the elimination of TCN-P from blood best fitted a single line with a mean half-life of 89.2 hours. Total-body blood clearance of TCN-P was 2.6 ml/minute/m². All patients had some TCN-P in plasma in the first 4 hours after administration (0.2–1.5 μg/ml), but the levels were variable and exhibited no consistent pattern. TCN-P was present at concentrations of around 0.5

TABLE 1.—Pharmacokinetics of TCN-P and TCN in phase I patients*

Patient No.	Dose (mg/m²/day)	TCN-P in blood			TCN in plasma	
		C_0 (μg/ml)	α-half-life (hrs)	Day 5 before treatment (μg/ml)	C_{plat} (μg/ml)	Day 5 before treatment (μg/ml)
1	24	7.2	2.5	7.1	0.02	0.03
2	24	5.3	7.4	4.3	0.32	0.30
3	24	4.9	7.2	1.4	0.00	0.15
4	36	7.1	6.6	ND	0.19	ND
5	36	6.2	6.5	3.9	0.07	0.05
6	36	6.5	8.6	9.3	0.25	0.34
7	44	12.1	6.5	6.3	0.07	0.19
8	44	8.4	8.4	2.5	0.27	0.21
9	55	6.9	5.5	6.5	0.34	0.46
10	55	10.0	4.3	4.8	0.41	0.69
11	55	6.0	6.3	ND	0.00	ND
12	55	5.1	3.4	4.6	0.16	0.14
Mean ± SEM		7.1 ± 0.6	6.1 ± 0.5	5.1 ± 0.7	0.18 ± 0.05	0.26 ± 0.06

*C_0 = concentration of TCN-P in blood immediately after iv injection; α-half-life = blood half-life over the first 6 hrs after administration; Day 5 before treatment = concentration on Day 5 immediately before the last dose of TCN-P; C_{plat} = plateau concentration of TCN in plasma over 0–6 hrs; and ND = not determined.

μg/ml in the plasma of one patient (No. 17) throughout the 3-day study, but could not be detected in the plasma of any of the other patients after about 4 hours. Plasma TCN concentrations after the first 4 hours were relatively constant over 3 days, with plateau concentrations up to 0.4 μg/ml.

No relationship was seen between the response or clinical toxicity of TCN-P and TCN-P pharmacokinetic parameters for any of the patients in the phase II study.

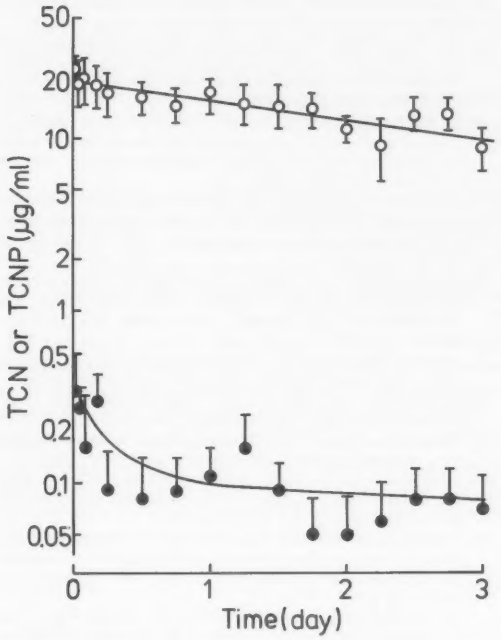

FIGURE 3.—TCN-P and TCN in blood plasma of patients receiving TCN-P (250 mg/m²). ○ = TCN-P in whole blood; ● = TCN in plasma. TCN-P could not be consistently detected in plasma. Values = mean of 6 patients. Bars are SE of mean.

DISCUSSION

Previous studies have shown that TCN-P is rapidly accumulated by rbcs, (7,11). This has been confirmed in the present study where TCN-P was present at high concentrations in whole blood, was barely detectable in plasma a few hours after the administration of the high dose of TCN-P (250 mg/m²), and was not detected at all at doses of TCN-P of 24–55 mg/m². TCN-P is first dephosphorylated to TCN before entry into cells (5). Human rbcs lack ecto-5′-nucleotidase (6) and conversion of TCN-P to TCN is catalyzed by plasma enzymes (7) or by cellular ecto-5′-nucleotidase at other sites in the body (6). Once inside the rbc TCN is phosphorylated to TCN-P, probably by adenosine kinase (5).

The initial half-life of TCN-P in the blood of phase I patients was 6.1 hours (range, 2.5–8.6). A second slower phase of elimination was seen but could not be characterized because of the short sampling time in the phase I studies. In the phase II patients studied over a longer time, the half-life of TCN-P in the blood was 89.2 hours (range, 31.2–221.5). In phase I patients, Lu et al (12) have reported a half-life of TCN-P in the blood of 28 hours when TCN-P was administered by continuous 5-day infusion at doses of 30–40 mg/m². The investigators also reported TCN-P in the plasma at steady-state concentrations up to 1 μg/ml. Crabtree et al (11) found very low concentrations of TCN-P in plasma when TCN-P was administered by 30-minute infusion to phase I patients at doses up to 92 mg/m². The reason for the difference in

TABLE 2.—Pharmacokinetics of TCN-P and TCN in phase II patients*

Patient No.	TCN-P in blood: Half-life (hrs)	Cl (ml/min/m²)	C_o (μg/ml)	TCN in plasma C_{plat} (μg/ml)
13	31.2	2.3	39.4	0.26
14	56.0	4.0	12.8	0.10
15	48.8	5.0	11.9	0.10
16	108.3	1.0	26.3	0.14
17	69.6	2.3	18.1	0.04
18	221.5	1.0	12.9	0.07
Mean ± SEM	89.2 ± 28.5	2.6 ± 0.6	20.2 ± 4.4	0.12 ± 0.03

*Half-life = blood half-life measured over 3 days; Cl = total-body blood clearance; C_o = concentration in blood immediately after iv injection; and C_{plat} = plateau concentration of TCN in plasma over 3 days.

plasma TCN-P concentrations when TCN-P is administered by rapid infusion and continuous infusion is not known. In the present study, there was no relationship between the dose of TCN-P administered and the concentration of TCN-P in the blood. Furthermore, TCN-P did not accumulate in the blood over 5 days of administration in phase I studies despite the long half-life of TCN-P in blood. These results suggest that uptake of TCN by rbcs or their capacity to retain TCN-P might be saturated at higher levels of TCN-P. Plasma was found to contain low but maintained concentrations of TCN, up to 0.4 μg/ml, which was probably formed by slow release of TCN-P from rbcs or other tissues and dephosphorylation by plasma or tissue ecto-5′-nucleotidase (6). Plasma concentrations of TCN up to 0.5 μg/ml were reported in the study by Lu et al (12). There have been reports of oxidation products formed from TCN by aged human erythrocytes in vitro (13), but no other metabolites of TCN-P, apart from TCN, were detected in the blood or plasma in the present study. Lu et al (12) have suggested that high plasma concentrations of TCN might correlate with liver toxicity of TCN-P and that persistence of TCN-P in rbcs is associated with iatrogenic anemia. We found no association between plasma TCN or blood TCN-P pharmacokinetics and hepatotoxicity, anemia, or any toxicity of TCN-P in the six patients in the phase II study.

In summary, the disposition of TCN-P has been studied in patients receiving TCN-P in phase I and II trials. TCN-P was rapidly accumulated by rbcs and had a terminal half-life in blood of 89 hours. TCN-P concentrations in blood did not correlate with the dose of TCN-P given and TCN-P did not accumulate in blood over 5 days of administration. TCN-P could not be consistently detected in plasma but plasma did contain low, maintained concentrations of TCN of up to 0.4 μg/ml. No correlation was found between TCN-P pharmacokinetic parameters and toxicity of TCN-P.

REFERENCES

1. Clinical Brochure. Tricylic nucleoside-5′-phosphate (NSC-280594). Bethesda, MD, National Cancer Institute, 1982.
2. ROTI-ROTI LW. 5′-Phosphate of tricyclic nucleoside: metabolism and cytotoxicity in L1210 cell cultures. Proc Am Assoc Cancer Res and ASCO 18:219, 1977.
3. ROTI-ROTI LW, ROTI-ROTI JL, and TOWNSEND LB. Studies on the mechanism of cytotoxicity of the tricyclic nucleoside NSC 154020 in L1210 cells. Proc Am Assoc Cancer Res and ASCO 19:40, 1978.
4. BENNETT LL, SMITHERS D, HILL DL, ET AL. Biochemical properties of the nucleoside of 3-amino-1,5-dihydro-5-methyl-1,4,5,6,8-pentaazaacenenaphthylene (NSC 154020). Biochem Pharmacol 27:233–241, 1978.
5. PLAGEMANN PGW. Transport, phosphorylation and toxicity of a tricyclic nucleoside in cultured Novikoff rat hepatoma cells and other cell lines and release of its monophosphate by the cells. J Natl Cancer Inst 57:1283–1295, 1976.
6. WOTRING LL, TOWNSEND LB, CRABTREE GW, ET AL. A possible role of ecto-5′-nucleotidase in cytotoxicity and intracellular nucleotide formation from tricyclic nucleoside-5′-phosphate (TCN-P). Proc Am Assoc Cancer Res 22:257, 1981.
7. BASSECHES PJ, DURSKI A, and POWIS G. High-performance liquid chromatographic assay of the antineoplastic agent tricyclic nucleoside-5′-phosphate and its disposition in rabbit. J Chromatogr 233:227–234, 1982.
8. SCHILCHER R, HAAS C, YOUNG J, ET AL. Phase I evaluation and pharmacokinetics of tricyclic nucleoside 5′-phosphate (TCN-P, NSC 2800594) in a weekly iv schedule. Proc Am Assoc Clin Oncol 2:31, 1983.
9. METZLER CM, ELFRING G, and MCEWEN AJ. A package of computer programs for pharmacokinetic modelling. Biometrics 30:3, 1974.
10. WAGNER JG. Linear pharmacokinetic equations allowing direct calculation of many needed pharmacokinetic parameters from the coefficients and exponents of polyexponential equations which have been fitted to the data. J Pharmacokinet Biopharm 4:443–467, 1976.
11. CRABTREE W, SPREMULLI EN, WIEMANN MC, ET AL. Phase I clinical and pharmacokinetic study of tricyclic nucleoside-5′-monophosphate (TCN-P, NSC 280594). Proc ASCO 2:32, 1983.
12. LU K, SAVARAJ N, FEUN LG, ET AL. Clinical pharmacology of 3-amino-1,5-dihydro-5-methyl-1-β-D-ribofuranosyl-1,4,5,6,8-pentaazaacenaphtylene-5′-monophosphate (TCN-P, NSC 280394). Proc ASCO 2:34, 1983.
13. SCHWEINSBERG PD, SMITH RG, and LOO TL. Identification of the metabolites of an antitumor tricyclic nucleoside (NSC-15402). Biochem Pharmacol 30:3521, 1981.

Phase I–II Study of Continuous-Infusion High-Dose Human Lymphoblastoid Interferon and the In Vitro Sensitivity of Leukemic Progenitors in Nonlymphocytic Leukemia[1,2]

Joseph Mirro,[3,*] Lois W. Dow,[3] David K. Kalwinsky,[3] Gary V. Dahl,[3] Phillip Weck,[4] John Whisnant,[4] and Sharon B. Murphy[3,5]

Twelve pediatric patients with nonlymphocytic leukemia were treated for 10 days with high-dose (15, 20, or 30 million U/m²/day) human lymphoblastoid interferon (Wellferon) administered by continuous iv infusion. Nine children had acute nonlymphocytic leukemia (ANLL) in relapse, two had Philadelphia chromosome-positive chronic myelocytic leukemia in myeloblastic crisis, and one had juvenile chronic myelocytic leukemia. Blast cell counts in the peripheral blood decreased in five patients with ANLL treated with the higher interferon doses; however, there was no evidence of an antileukemic effect in the marrow. Dose-limiting toxicity, which included malaise, hepatotoxicity, and coagulation abnormalities, was observed in patients given 20 or 30 million U/m²/day. Studies of the growth of leukemic progenitor cells in vitro in the presence of interferon disclosed a concentration-related inhibition of colony formation. Patients who had a decrease in peripheral blast cell counts demonstrated greater in vitro inhibition of clonogenic leukemic progenitors than patients whose blast cell counts did not decrease. However, the serum interferon concentrations in patients given clinically tolerable doses were lower than those concentrations which inhibited leukemic cell growth in vitro by a median of 42% (1000 U/ml). This study failed to demonstrate clinically significant antileukemic activity against nonlymphocytic leukemia in patients given high-dose constant-infusion interferon, and the toxicity of this approach was prohibitive. [Cancer Treat Rep 70:363–367, 1986]

Interferon is a naturally occurring protein with potent antiviral, immunologic, and antiproliferative properties (1,2). Early clinical studies of interferon were limited by the lack of sufficient quantities of the agent for reliable testing. The recent availability of large quantities of relatively pure interferon from cultured human cells (3) allowed us to conduct this phase I–II study in children with nonlymphocytic leukemia. Continuous high-dose infusions of interferon were used because of reports indicating that therapeutic effects, and serum interferon concentrations causing 50% inhibition of myeloblast growth in vitro, could be obtained (4,5). Additionally, if the antiproliferative activity of interferon was directly responsible for its clinical effects, we might improve results by administering high doses as a prolonged continuous infusion (6–9). We planned to test this hypothesis by comparing clinical responses with serum interferon concentrations that inhibited leukemic progenitor cell growth in vitro.

MATERIALS AND METHODS

Patients

In this phase I–II trial, 12 patients (1.7–19.5 years of age) with myeloid leukemia refractory to conventional therapy were treated with constant infusions of human lymphoblastoid interferon (Wellferon, Burroughs Wellcome Co). All patients entered on this therapeutic trial are included in this report. Nine had acute nonlympho-

[1]Received Apr 25, 1985; revised July 8, 1985; accepted July 29, 1985.

[2]Supported by Public Health Service grants RR-05584 (Division of Research Resources) and CA-20180 (National Cancer Institute) from the National Institutes of Health, Department of Health and Human Services, and by funds from the Burroughs Wellcome Co and the American Lebanese Syrian Associated Charities. J. Mirro is the recipient of a Clinical Oncology Career Development Award from the American Cancer Society.

[3]Department of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN.

[4]Burroughs Wellcome Co, Research Triangle Park, NC.

[5]We thank M. Nash for technical, J. Gilbert for editorial, and J. Harber for statistical assistance.

[*]*Reprint requests to:* Joseph Mirro, Jr, MD, Department of Hematology/Oncology, St. Jude Children's Research Hospital, 332 N Lauderdale, Memphis, TN 38101.

cytic leukemia (ANLL), two had Philadelphia chromosome-positive chronic myelocytic leukemia in myeloblastic crisis (CML-BC), and one had juvenile chronic myelocytic leukemia (JCML), a rare disorder poorly responsive to conventional therapy. All patients had recovered from previous therapy and had normal renal and hepatic functions prior to study entry. This study was approved by the institution's Clinical Trials Committee and informed consent was obtained for all patients.

Treatment

The lymphoblastoid interferon Wellferon is a purified preparation of alpha interferons from the cultured human lymphoblastoid cells Namalwa. These cells are induced with Sendai virus, resulting in synthesis and secretion of interferons. Wellferon is isolated, concentrated, and purified from cell-free supernatants to a level of 2×10^8 IU of human alpha interferon per milligram of protein. This human lymphoblastoid interferon is therefore > 90% pure and contains at least eight human alpha interferon subtypes (3). The biological activity of Wellferon is expressed in megaunits (10^6 IU = 1 megaunit) and is determined in a viral cytopathic effect inhibition assay, standardized against the international standard MRC-69/19.

Each patient received a 10-day continuous iv infusion of interferon. Criteria for discontinuing therapy included hypotension, severe somnolence or seizures, an increase in the SGOT to greater than three times pretreatment values, or a bilirubin > 4 mg/100 ml. The first two patients received 30 megaunits/m^2/day; however, this dose produced unacceptable toxicity, requiring a 50% dose reduction to 15 megaunits/m^2/day for the next five children. Since the lower dose of 15 megaunits/m^2/day was found to be clinically tolerable, we planned a dose escalation, until limited by toxicity, of 5 megaunits/m^2/day for each group of five patients subsequently entered on this protocol.

Clinical and Laboratory Evaluation

Clinical evaluation was performed daily, and the anticipated symptomatic interferon toxicity was graded using a scale which ranged from mild toxicity (grade 1) to severe toxicity (grade 3). Daily laboratory studies included a cbc, liver and renal function tests, prothrombin time, activated partial thromboplastin time, and fibrinogen. Laboratory toxicity was graded based on a standard scale used by the Pediatric Oncology Group. Bone marrow was evaluated immediately before and after the 10-day infusion and, in some patients, 2 weeks later. The marrow evaluation and blood cell counts were adequate to assess response in all patients.

Steady-state serum interferon concentrations were determined on Days 2, 5, and 10 in a microtiter assay that measured the inhibition of cytopathic effects produced in FS-7 cells by vesicular stomatitis virus. One unit of interferon activity is the reciprocal of the serum dilution resulting in 50% inhibition of cytopathic effect, with standardization against the National Institutes of Health reference (GO23-901-527).

Prior to interferon treatment, low-density marrow or blood mononuclear cells (< 1.077 g/ml) from each patient were cultured in 0.3% agar in McCoy's medium with 20% fetal calf serum over leukocyte feeder layers (10). Cells were cultured at 37°C in 7.5% CO_2 in medium without interferon or with increasing concentrations of the agent (100, 1000, 2000, or 4000 U/ml). Leukemic colony-forming units were scored as clusters (3-19 or 20-50 cells) and colonies (> 50 cells) on Day 7. The cluster and colony morphology for these patients was similar to comparable patients from which cytogenetic studies have confirmed in vitro growth of leukemic progenitors (L. W. Dow, unpublished observation).

RESULTS

Blast cell counts in peripheral blood increased in all five patients receiving 15 megaunits/m^2/day; the median increase was 145% (range, 75%-998%). By contrast, three of the five patients receiving 20 megaunits/m^2/day and both of those receiving 30 megaunits/m^2/day had decreased circulating blast cell counts on Day 10, indicative of interferon-mediated antiproliferative effects (table 1). None of the patients showed evidence of a reduction in marrow blast cell counts or cellularity (data not shown). Although we cannot exclude a 20% response rate because of the small number of patients on this study, this dose schedule resulted in unacceptable toxicity and, therefore, this trial was closed.

Interferon serum concentrations were dose-related, but exhibited interpatient variability. Steady-state concentrations on Day 10 ranged from 78 to 363 U/ml (median, 139) in patients receiving 15 megaunits/m^2/day, but were slightly higher in those receiving 20 megaunits/m^2/day (Wilcoxon, rank-sum test; $P = 0.075$). The two children given 30 megaunits/m^2/day achieved concentrations of 2050 and 1190 U/ml (table 1).

Interferon caused dose-related inhibition of leukemic progenitor colony and cluster formation in vitro. Cells from nine patients (six with ANLL, two with CML-BC, and one with JCML) grew well enough (50-1682 clusters and colonies per 2×10^5 cells) to permit an evaluation of the antiproliferative activity of interferon. Figure 1 demonstrates the agent's dose-related inhibition of colony formation. The addition of 100 U/ml of interferon resulted in < 25% inhibition of cluster and colony formation by cells from seven of nine patients (median, 19%). Higher concentrations of 1000 U/ml produced 2%-100% inhibition (median, 42%), 2000 U/ml produced 29%-100% (median, 54%), and 4000 U/ml produced 33%-100% (median, 70%). A binomial test was used to analyze for a significant decrease in leukemic colony forma-

TABLE 1.—Clinical characteristics and treatment responses

Patient No.	Age (yrs)/sex	Diagnosis	Interferon dose (megaunits/m^2/day)	Steady-state interferon serum concentration* (U/ml)	Absolute Nos. of blast cells in peripheral blood (× 10^6/L)	
					Preinfusion	Day 10
1	17.0/F	ANLL	30	2050	7524	340
2	8.5/M	ANLL	30	1190	16,198	10,153
3	13.1/F	ANLL	20	108	3500	189
4	10.1/F	ANLL	20	352	252	0
5	17.5/M	ANLL	20	1024	44	0
6	4.2/F	ANLL	20	384	28,650	42,997
7	3.2/M	ANLL	20	768	0	0
8	1.7/M	JCML	15	124	315	552
9	10.0/F	CML-BC	15	78	47,625	306,900
10	14.5/M	ANLL	15	363	384	4218
11	19.5/M	CML-BC	15	190	91,500	210,000
12	14.3/M	ANLL	15	139	138	336

* Day 10 concentrations (not significantly different from Day 2 or 5 concentrations).

tion between different levels of interferon. A significant decrease in colony formation occurred between concentrations of 100 and 1000 U/ml ($P = 0.002$) and 1000 and 2000 U/ml ($P = 0.004$); however, there was not a significant decrease when 100 U/ml was added ($P = 0.09$) or between the concentrations of 2000 and 4000 U/ml ($P = 0.188$).

Statistical correlation between serum interferon levels, inhibition of leukemia progenitors, and a decrease in the peripheral blast count were limited by the small number of patients in this trial. However, of the five patients (Nos. 1-5) who demonstrated decreases in peripheral blast cell counts, leukemic progenitor cells from three (Nos. 1-3) were tested for sensitivity to interferon in vitro. Leukemic progenitors from these three patients showed a significantly increased cell kill in vitro by 1000 and 2000 U/ml of interferon when compared to cells from the five nonresponding patients with ANLL or CML-BC (Wilcoxon; $P = 0.04$ at 1000 and 2000 U/ml). If Patient 8 with JCML (a disease generally resistant to therapy) is included in the analysis, the difference between these groups is significant only at an interferon concentration of 2000 U/ml ($P = 0.03$).

Toxicity

Symptomatic toxicity was generally severe and is summarized in table 2. Fever developed in all patients (maximum temperature range from 38.7°C to 41.2°C) and in four it persisted throughout the interferon infusion. Tachyphylaxis of fever occurred in eight patients. Eleven patients were treated with broad-spectrum iv antibiotics because they were severely neutropenic when they developed fevers > 38.5°C. Four patients had documented bacterial infections, which included two with pneumonia, one with cervical adenitis, and one with cellulitis. Interestingly, one patient developed an apparent viral pharyngitis on interferon. Fatigue and lethargy, usually progressive, were a consistent complication of treatment. This symptomatic toxicity was severe and resulted in almost complete bed rest (Karnofsky score < 50) in seven patients. Nausea occurred in ten patients and although only eight had associated vomiting, all ten required iv hydration. Chills noted in nine patients appeared to be dose-related and were only partially relieved by meperidine. Other symptomatic toxicity was mild and included headaches in six patients, erythema of the oral mucous membranes in five, and muscle aches in five. Hypotension developed in one of the two patients who received 30 megaunits/m^2/day; the other child had seizures on Day 9 and died the next day of an apparent intracranial hemorrhage.

A previously described coagulopathy that was usually not associated with overt bleeding occurred in nine patients (11). The activated partial thromboplastin time was prolonged in all nine patients, while the prothrombin time was prolonged in only four. Vitamin K administration improved but did not completely correct these coagulation abnormalities, suggesting that both vitamin K-dependent and K-independent factors were affected by interferon. Studies of coagulation factors confirmed low levels of the vitamin K-dependent factors (II, VII, IX, and X) and K-independent factor (XII). Antibiotics did not appear to be responsible for this coagulopathy, since it occurred in the one patient not receiving antibiotics and started in four others prior to antibiotic therapy.

Severe hepatotoxicity, demonstrated by an increase in liver enzymes greater than three times normal, occurred in one patient on Day 9 and in three patients after the completion of the planned 10-day infusion. This toxicity resulted in the discontinuation of one patient's infusion on Day 9. All four patients were receiving 20 megaunits/m^2/day, and although the bilirubin was not increased in any patient, the maximum SGOT concentrations ranged from 187 to 695 IU/L (normal, < 35) and SGPT ranged from 86 to 414 IU/L (normal, < 35). The liver enzyme abnormalities resolved within a few days. Hematopoietic toxicity could not be adequately assessed

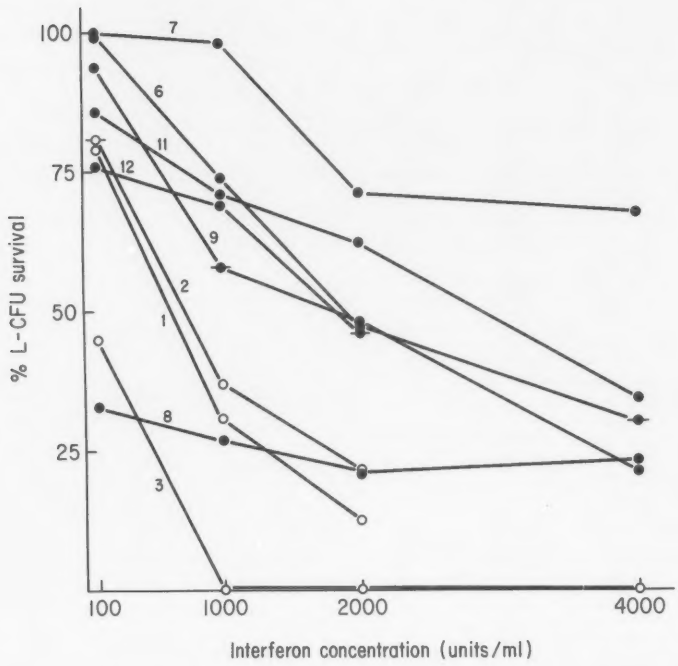

FIGURE 1.—Growth inhibition of leukemic progenitor cells (L-CFU) in vitro by human lymphoblastoid interferon (Wellferon). Individual patient results are numbered. Patients demonstrating a decrease in the peripheral blast cell count are indicated by ○; those showing no change or an increase are indicated by ●. The median % of leukemic progenitor survival is indicated by ———.

in this study, since all patients had marrow replacement by leukemic cells.

DISCUSSION

Early clinical trials suggested that leukocyte interferon might be effective against nonlymphocytic leukemia (6-9). Some investigators using daily doses > 30 megaunits/m^2/day achieved serum concentrations of 1000 U/ml, which resulted in a 50% inhibition of leukemic cell growth in vitro (9). These trials suggested that such doses were clinically tolerable and resulted in significant clinical responses (7,9). In this phase I-II study, we attempted to test the effects of human lymphoblastoid interferon in children with nonlymphocytic leukemia in relapse. We assumed that the antiproliferative activity of interferon would be more important than its immunomodulating effects, since patients in relapse would not have normal immunologic responses. In vitro studies of the patients' leukemic marrow cells confirmed the ability of interferon to inhibit leukemic cell growth (4,5). However, concentrations of 1000 and 2000 U/ml resulted in median growth inhibitions of only 42% and 54%, respectively. Furthermore, the serum concentrations of interferon achieved in patients were dose-related. At doses we considered clinically tolerable (20 megaunits/m^2/day), concentrations were generally < 1000 U/ml.

Patients 1 and 2 received 30 megaunits/m^2/day of interferon and achieved serum concentrations of 1190 and 2050 U/ml which corresponded respectively to 63% and 87% in vitro growth inhibition of their leukemic progenitors. Although both patients had a decreased peripheral

TABLE 2.—Toxic effects of interferon in 12 pediatric patients with nonlymphocytic leukemia

Symptom or sign	No. of patients
Fever	12
Fatigue and lethargy	12
Nausea	10
Chills	9
Coagulopathy	9
Headache	6
Muscle ache	5
Erythema of oral mucous membranes	5
Liver enzyme abnormalities	4
Hypotension*	1
Seizures*	1

* Limited to patients who received 30 megaunits/m^2/day.

blast cell count, neither demonstrated a marrow response. Patient 3 received 20 megaunits/m^2/day, and despite a low serum level, had a decrease in peripheral blasts. This patient's leukemic progenitors appeared to be unusually sensitive in vitro, with 55% inhibition at 100 U/ml and complete inhibition at 1000 U/ml; however, this patient did not have a clinically significant response. Thus, although a correlation exists between decreasing peripheral blast counts and in vitro sensitivity, this correlation was not clinically meaningful in this trial, since no remissions were obtained.

The maximum tolerable dose in this study, 20 megaunits/m^2/day, was lower than tolerable doses identified in previous studies (7,9). Even at this dose, the symptomatic toxicity, coagulopathy, and hepatotoxicity were severe. One child's death (Patient 2) was possibly treatment-related, since this patient developed an interferon-induced coagulopathy with a low platelet count (10,000/mm^3) and had generalized seizures. He subsequently had rapid neurologic deterioration despite intensive support, and died the following day of presumed CNS hemorrhage; unfortunately, a postmortem examination was denied. Because of the toxicity and the lack of significant clinical responses, we conclude that interferon, as administered here, is not useful against childhood nonlymphocytic leukemia. Despite earlier suggestions of feasibility (4,7,9), direct antiproliferative effects were difficult to achieve in vivo.

Further studies are needed to assess the therapeutic value of interferon's immunomodulating effects. Additional studies are also required to evaluate if interferon is useful in controlling minimal residual disease. The concentration of interferon which inhibited leukemic cell growth in vitro was not clinically tolerable in this continuous-infusion dose schedule. Perhaps interferon given by bolus injection could, for brief periods, achieve cytotoxic levels for leukemic progenitor cells but remain clinically tolerable. Alternatively, other types of interferon (eg, recombinant or gamma) may be of some therapeutic benefit.

REFERENCES

1. BORDEN EC, and BALL LA. Interferon: biochemical cell growth inhibitory and immunological effects. *In* Progress in Hematology, vol XII (Brown ER, ed). New York, Grune and Stratton, 1981, pp 299-339.
2. GUTTERMAN JU, BLUMENSCHEIN GR, ALEXANIAN R, ET AL. Leukocyte interferon-induced tumor regression in human metastatic breast cancer, multiple myeloma, and malignant lymphoma. Ann Intern Med 93:399-406, 1980.
3. FINTER NB, and FANTES KH. The purity and safety of interferon prepared for clinical use: the case of lymphoblastoid interferon. *In* Interferon 2 (Gresser I, ed). London, Academic Press, Inc, 1980, pp 65-80.
4. BALKWILL FR, and OLIVER RTD. Growth inhibitory effects of interferon on normal and malignant human haematopoietic cells. Int J Cancer 20:500-505, 1977.
5. TAETLE R, BUICK RN, and MCCULLOCH EA. Effect of interferon on colony formation in culture by blast cell progenitors in acute myeloblastic leukaemia. Blood 56:549-552, 1980.
6. HILL NO, LOEB E, PARDUE AS, ET AL. Response of acute leukemia to leukocyte interferon. J Clin Hematol Oncol 9:137-149, 1979.
7. ROHATINER AZS, BALKWILL FR, GRIFFIN DB, ET AL. A phase I study of human lymphoblastoid interferon administered by continuous intravenous infusion. Cancer Chemother Pharmacol 9:97-102, 1982.
8. TALPAZ M, MCCREDIE KB, MAVLIGIT GM, ET AL. Leukocyte interferon-induced myeloid cytoreduction in chronic myelogenous leukemia. Blood 62:689-692, 1983.
9. ROHATINER AZS, BALKWILL F, and LISTER TA. Interferon in acute myelogenous leukemia: a preliminary report. *In* Modern Trends in Human Leukemia (Neth R, ed). Berlin, Springer-Verlag, 1983, pp 56-58.
10. MOORE MAS, WILLIAMS N, and METCALF D. In vitro colony formation by normal and leukemic human hematopoietic cells: characterization of the colony-forming cells. J Natl Cancer Inst 50:603-623, 1973.
11. MIRRO J, JR, KALWINSKY D, WHISNANT J, ET AL. Coagulopathy induced by continuous infusion of high doses of human lymphoblastoid interferon. Cancer Treat Rep 69:315-317, 1985.

Ethinyl Estradiol and Medroxyprogesterone Acetate in Patients With Epithelial Ovarian Carcinoma: A Phase II Study[1,2]

Ralph S. Freedman,[3,*] Patton B. Saul,[3] Creighton L. Edwards,[3] Christopher J. Jolles,[3,4] David M. Gershenson,[3] Lovell A. Jones,[3] E. Neely Atkinson,[3] and William J. Dana[3,5]

Patients with ovarian carcinoma refractory to chemotherapy received a sequential combination of ethinyl estradiol and medroxyprogesterone acetate in two dose regimens. There was no difference in therapeutic activity of the two dose regimens. Of 65 patients, nine (14%) responded to treatment and 13 (20%) had stable disease. Vascular complications occurred in three patients; hemiplegia developed in one of those. Nine patients had significant nausea and vomiting, and one experienced severe depression that required treatment withdrawal. The sequential and combined use of ethinyl estradiol and medroxyprogesterone acetate may provide an alternative treatment for certain patients with ovarian carcinoma that does not respond to optimum chemotherapy. Additional studies are required to determine if synergism exists between this treatment and other modalities of therapy. Further investigation is required into the vascular disorders that complicate therapy to determine whether appropriate preventive measures are possible. [Cancer Treat Rep 70:369–373, 1986]

Epithelial ovarian carcinoma remains a significant cause of cancer-related mortality despite the invaluable contributions of chemotherapy and radiation therapy. Patients whose diseases fail to respond to chemotherapy combinations that include cisplatin rarely respond to other chemotherapy agents (1,2). Reports on the use of several hormonal agents in ovarian cancer suggest that they have therapeutic activity, but remain anecdotal or unconfirmed (3–6). Some of the earlier studies are also difficult to interpret because the hormones may have interacted with previous therapy (5,7). More recent studies with medroxyprogesterone acetate (MPA) alone showed almost no activity against chemotherapy-refractory disease (8,9). Although an estrogen-progestogen combination has been used previously (5), our experience with a combination of ethinyl estradiol (EE) and MPA is the first report of a planned combination treatment approach in this disease.

The continued search for hormones active against ovarian carcinoma is supported by the knowledge that both estrogen receptors (ER) and progesterone receptors (PR) have been detected in epithelial ovarian malignancies, although PR are detected less frequently (10). There is a likelihood, therefore, that suitable hormone manipulations could accomplish activity against ovarian carcinoma similar to that observed against carcinomas of the breast (11) and endometrium (12). The relative deficiency of the PR could account for previous observed refractoriness to MPA, which is known to be active against other PR-positive malignancies (13). Estrogens have a high affinity for carcinomatous tissues and appear to facilitate the appearance of cytosol PR (14). We have postulated that the sequential and simultaneous administration of a potent estrogen and a progestogen may provide a therapeutic advantage over progestogens alone, possibly by inducing cytosol PR and increasing tumor responsiveness to therapy.

In a preliminary report (15), we documented the induction of PR in a vaginal metastasis that was ER-positive and PR-negative prior to therapy. Hamilton et al (16) have observed the induction of PR in a human ovarian carcinoma transplanted into a nude mouse with 17β-estradiol. Here we analyzed the activity and side effects observed in the first 65 patients treated with EE and

[1]Received Feb 7, 1985; revised June 21, 1985; accepted July 8, 1985.

[2]Supported in part by Public Health Service grant CA-31382 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Gynecology (*R. S. Freedman, P. B. Saul, C. L. Edwards, C. J. Jolles, D. M. Gershenson*, and *L. A. Jones*), Biomathematics (*E. N. Atkinson*), and Pharmacy (*W. J. Dana*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]*Present address:* University of Utah Medical Center, Department of Obstetrics and Gynecology, Division of Gynecology Oncology, Salt Lake City.

[5]We thank Wanza Scott for her assistance.

[*]*Reprint requests to:* Ralph S. Freedman, MD, PhD, Department of Gynecology, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

in a phase II study design.

MATERIALS AND METHODS

Patients with epithelial ovarian carcinoma refractory to chemotherapy that usually included cisplatin were entered in the study if they had an expected survival of 2-3 months. Patients were excluded if they had received previous hormone therapy. All patients were registered and received the combination of EE and MPA.

The first group of patients (Group A) received EE at a dose of 100 μg/day on Days 1-25 and MPA at a dose of 50 mg twice a day on Days 8-25. No treatment was administered on Days 26-30, and then the cycle was repeated. After the first 37 patients had been treated, a second protocol was started on another group of patients (Group B) with the dose of EE reduced to 50 μg/day and the dose of MPA increased to 50 mg four times a day. The treatment schedule remained the same. The changes in dosage were introduced in an effort to reduce side effects observed and attributed to EE in Group A patients and to determine whether a further increase in the observed response rate was attainable by increasing the dose of MPA.

Tumor specimens were available for ER analysis in 17 patients, and in eight of these patients PR could be done. Specimens were collected and analyzed as described previously (15).

Response criteria used in the clinical analysis were based on World Health Organization recommendations (17). Briefly, a complete response (CR) was defined as the complete disappearance of a clinically or radiologically documented lesion for at least 4 weeks. Partial response was defined as a 50% reduction in the sum of the product of the maximum perpendicular diameters for a minimum of 4 weeks and the absence of progression at other sites. Stable disease was defined as no change or a < 50% reduction in tumor diameters for a minimum of 8 weeks. The Kaplan-Meier method was used for survival rate analysis (18). It is estimated that the chemotherapy response rate is 5% for patients failing first-line chemotherapy for ovarian carcinoma. A sample size of 30 was chosen, since the figure gives a power of 75% (assuming a significance level of 5%) against an increase in the rate to 20%. Additional patients up to 65 were entered to test the hypothesis that a higher dose of MPA might increase the response rate.

Characteristics of the 65 patients studied are shown in table 1. The mean age of the group is 56 years (range, 27-83); however, 17 patients were < 50 years of age and therefore unlikely to be premenopausal. Serous carcinomas were the most common type of tumor, but ten patients had endometrioid tumors. These carcinomas are similar in appearance to endometrial carcinomas and might be expected to share the characteristic of hormone responsiveness.

All patients had received adequate first-line chemotherapy. Sixty-two patients received cisplatin. Forty-five patients had received two or more types of adjuvant therapy.

TABLE 1.—Clinical characteristics of study patients

Characteristic	No. of patients
Stage*	
II	4
III	40
IV	16
Unknown	5
Histology	
Serous	37
Endometrioid	10
Other (mucinous clear cell, undifferentiated adenocarcinoma, and mixed)	18
Grade (pattern method)	
I	7
II	17
III	34
Ungraded	7
Previous treatment	
Surgery	65†
Chemotherapy with cisplatin	63
Radiotherapy	14
Treatment No.	
1	20
2	30
3	15
Previous positive treatment response	23
Total	65

*Disease stage was evaluated according to the standards of the International Federation of Gynecology and Obstetrics.
†Two patients had biopsy alone.

Almost all patients had symptoms related to their malignancy, which were considered easily evaluable in 62 patients. Three patients had small-volume intra-abdominal disease and were therefore less easy to evaluate.

RESULTS

The numbers of patients who demonstrated a response or had stable disease or disease progression at each dose level are shown in table 2. Response rates for patients who completed a minimum of one course of EE-MPA are also provided.

Thirty-seven patients were in Group A and 28 were in Group B. There were no statistical differences between Groups A and B in terms of response rates. Data on response and toxicity are therefore presented for the entire group. The tumors of nine of 65 patients (14%) responded to treatment (95% confidence interval, 8%-24%). Twenty percent of the patients had stable disease and 66% had progressive disease. Fifty-one patients completed at least one course of treatment. The median survival time for the entire group was 5.2 months.

The characteristics of the diseases that responded are shown in table 3. All patients had easily evaluable disease, including one with a measurable and biopsy-con-

TABLE 2.—Response to EE and MPA

Treatment	No. of patients	Response (%)			
		Complete	Partial	Stable disease	Progressive disease
Group A	37	1 (3)	5 (13)	7 (19)	24 (65)
Group B	28	0	3 (11)	6 (21)	19 (68)
Total No. registered and treated	65	1 (2)	8 (12)	13 (20)	43 (66)
Group A	30	1 (7)	5 (17)	7 (23)	17 (57)
Group B	21	0	3 (14)	6 (29)	12 (57)
Total No. registered and treated	51*	1 (4)	8 (16)	13 (25)	29 (57)

*Fourteen patients were unable to complete 1 course because of toxicity or disease progression.

firmed parenchymal lung metastasis. This patient's disease responded partially to treatment according to a follow-up computerized tomography (CT) scan evaluation, which showed persistence of mediastinal lymphadenopathy although the parenchymal lung lesion remained in CR. Information is also provided on the duration of response, survival, number of treatment courses received by each patient, and subsequent treatment.

Three patients had prolonged stable disease after receiving EE and MPA and warrant further desciption. The first patient was 69 years of age at entry in the study and initially presented with a stage IV, grade III serous carcinoma. Combination chemotherapy that included cisplatin was administered, but second-look laparotomy showed sizable liver metastasis and peritoneal implants. The liver metastasis underwent calcification and remained stable for 20 months, after which disease progression in the liver and at other sites was noted.

The second patient was 58 years of age and presented with stage IV serous carcinoma consisting of abdominopelvic masses and pulmonary metastases. The patient had received cisplatin and doxorubicin followed by two other chemotherapy regimens. Treatment was followed by progression of the abdominal and pulmonary lesions. After ten courses of EE and MPA, the abdominal tumor softened and the pulmonary lesions stabilized. After 9 months the patient was re-explored and tumor-reductive surgery was performed. The patient remained on EE and MPA and continued to have stable disease for an additional 10 months. After 19 months of treatment, progression of the radiologically detectable chest lesions occurred.

The third patient was 69 years of age and presented with stage III, grade III serous carcinoma. She had received cisplatin and an alkylating agent after tumor-reductive surgery and was re-explored. A jejunal obstruction from an invading carcinoma was found. The tumor was excised and residual disease was found to be < 2 cm in diameter. The patient remained free of disease for 22 months. Repeated clinical examinations and CT scans have been negative.

Receptor Studies

Receptor information is shown in table 4. Only two of the nine responsive tumors had receptor data; both had increased ER and PR levels. In addition, there were three patients with receptor data who had prolonged stable disease, two of whom had ER levels > 10 fmols/mg of receptor protein. One of these three patients was previously reported to show induction of PR in a surface-growing vaginal metastasis following EE and MPA (16). A second patient with a jejunal metastasis (described above) showed ER and PR values of 25.9 and 71.6 fmols/mg of receptor protein, respectively.

Toxicity

Table 5 shows patients' reactions to drug toxicity. Hemiparesis from cerebrovascular occlusion occurred in one patient after two treatment courses and was considered a probable consequence of hormone therapy. The patient subsequently recovered from the hemiparesis, but slow progression of the tumor was apparent. This complication occurred at an EE dose of 50 μg. Another patient developed a transient cerebral ischemic attack and presented with dysarthria at a 100-μg dose of EE. Long saphenous phlebitis occurred in a third patient. Since this patient had experienced a partial response to the hormones, treatment was continued under full anticoagulation therapy. Depression and headaches were severe enough to require withdrawal of treatment in one patient. This patient had a complete neurological evaluation, but no focal organic disease could be found. Nausea and vomiting occurred in nine patients, but it was difficult to determine whether the hormone treatment was primarily responsible or whether intestinal dysfunction associated with disease progression was contributory. These nine patients were among the group that was unable to complete a single course of treatment

DISCUSSION

This study established that a combination of EE and MPA is active in epithelial ovarian carcinoma refractory

TABLE 3.—Clinical characteristics of c

Patient age (yrs)	Histopathology/ grade/stage	Prior treatment	Response to prior treatment†	Tumor status	Type of response	Response duration (mos)
59	A/I/III	S + P	+	Pelvic mass	Partial	4
55	EC/II/III	S + P	–	Pelvic mass	Partial	1
64	Se/II/III	S + P	–	Abdominal (negative CT and biopsy confirmed)	Complete	12
66	Se/III/IV	S + P	+	Abdominal/pelvic mass (negative CT confirmed)	Partial	3
56	Se/II/III	B + P	+	Pelvic mass, ascites, supraclavicular node	Partial	2
72	Se/III/IV	S + P	+	Pelvic mass	Partial	> 12
59	E/II/III	S + HAC	+	Pulmonary/mediastinal mass (negative CXR, CT, and biopsy confirmed)	Partial	> 8
48	Se/III/III	S + P	–	Pelvic mass	Partial	> 2
74	Se/III/III	S + P	–	Pelvic/umbilical mass	Partial	> 5

* A = adenocarcinoma; E = endometrioid; C = clear cell; Se = serous; S = tumor-reductive surgery; and CXR = chest radiograph.
† + = previous response; – = no previous response.

istics of disease that responded to treatment*

Response duration (mos)	Survival (mos)	No. of courses	Side effects	Comments
4	13	5	—	ER, 153.3 fmols/mg of receptor protein/PR, 100.3 fmols/mg of receptor protein; no response to subsequent chemotherapy
1	6	4	—	—
12	18	11	Vaginal bleeding	No response to subsequent chemotherapy
3	13	2	Tremors, headaches, depression	Partial response with cyclophosphamide
2	3	2	—	—
> 12	> 12	> 12	Phlebitis	ER, 32.5 fmols/mg of receptor protein/PR, 109.5 fmols/mg of receptor protein
> 8	> 8	> 8	—	—
> 2	> 2	> 2	—	—
> 5	> 7	6	—	Partial response with radiotherapy

e surgery; P = cisplatin; B = biopsy; HAC = hexamethylmelamine, doxorubicin, and cyclophosphamide;

TABLE 4.—ER and PR and relationship to response

Responders (fmols/mg of ER/PR protein)	Nonresponders	
	> 10 fmols/mg of ER/PR protein	< 10 fmols/mg of ER/PR protein
32.5/109.5	13.2/6.1*	5.7/< 5
135.3/100.3	18.2/-	1.9/< 5
	48.81/-	1/11.5
	19.2/-	9.7/< 5
	13.3/-	< 1.0/86*
	25.9/71.6†	1.45/-
		7.9/-
		2.2/-
		< 1.0/-
		0.8/-

*Stable disease for 6 mos.
†Stable disease for 22 mos.

to previous chemotherapy. CR or partial response in nine patients and stable disease in 13 patients were achieved with this combination. Several patients in the latter group had long survival patterns that may have been influenced by the hormone administration.

The study may be compared with an earlier one in which oral MPA was used alone to treat patients with recurrent and metastatic ovarian carcinoma (8). Only one of 19 patients responded in this study. Disease in patients < 48 years of age or disease in an advanced stage appeared to be less likely to respond to hormone manipulation than in older patients, but histologic pattern did not appear to determine response to therapy. In older patients hormone therapy is more effective against epithelial ovarian carcinoma as in the case of breast cancer (11).

Receptor information did not allow us to conclude that response to treatment was mediated through an increase in PR, although two patients among the responders had receptor data available which were elevated in both patients and paralleled the experience in breast cancer.

The vascular system appeared to be most affected by the drugs' toxic effects. This problem could possibly be aggravated by an inherent hypercoagulable state in some patients. It is uncertain whether concurrent medication with anticoagulants might be effective as thromboembolism prophylaxis. This problem is being investigated further in collaboration with our hematology colleagues.

We consider that the sequential and combined administration of EE and MPA may offer an alternative to patients with ovarian carcinoma that does not respond to optimum first-line chemotherapy. Older patients and those without ascites or intestinal dysfunction appear to be better candidates for the treatment. Further studies should be undertaken before this hormone combination can be added to first-line chemotherapy regimens.

MPA in 50-mg tablets was supplied by the Upjohn Company, Kalamazoo, MI.

TABLE 5.—Toxicity

Toxic effect	No. of patients	
	Group A	Group B
None	28	24
Nausea and vomiting	6	3
Phlebitis	1	
Hemiparesis		1
Transient cerebral ischemia-dysarthria	1	
Depression	1	

REFERENCES

1. EDWARDS CL, HERSON J, GERSHENSON DM, ET AL. A prospective randomized clinical trial of melphalan and *cis*-platinum versus hexamethylmelamine, adriamycin, and cyclophosphamide in advanced ovarian cancer. Gynecol Oncol 15:261-277, 1983.
2. NEIJT JP, VAN DER BURG MEL, VRIESENDORP R, ET AL. Randomized trial comparing two combination chemotherapy regimens (HEXA-CAF vs CHAP-5) in advanced ovarian carcinoma. Lancet 2:594-600, 1984.
3. LONG RTL, and EVANS AM. Diethylstilbestrol as a chemotherapeutic agent for ovarian carcinoma. Mo Med 60:1125-1127, 1963.
4. TIMOTHY I. Progestogen therapy for ovarian carcinoma. Br J Obstet Gynaecol 89:561-563, 1982.
5. WARD HWC. Progestogen therapy for ovarian carcinoma. J Obstet Gynaecol Br Commonwealth 79:555-559, 1972.
6. GEISLER HE. Megestrol acetate for the palliation of advanced ovarian carcinoma. Obstet Gynecol 61:95-98, 1983.
7. JOLLES B. Progesterone in the treatment of advanced malignant tumours of breast, ovary and uterus. Br J Cancer 16:209-211, 1962.
8. MALKASIAN GD, JR, DECKER DG, JORGENSEN EO, ET AL. Medroxyprogesterone acetate for the treatment of metastatic and recurrent ovarian carcinoma. Cancer Treat Rep 61:913-914, 1977.
9. SLAYTON RE, PAGANO M, and CREECH RH. Progestin therapy for advanced ovarian cancer: a phase II Eastern Cooperative Oncology Group trial. Cancer Treat Rep 65:895-896, 1981.
10. JONES LA, EDWARDS CL, FREEDMAN RS, ET AL. Estrogen and progesterone receptor titers in primary epithelial ovarian carcinomas. Int J Cancer 32:567-571, 1983.
11. MCGUIRE WL, HORWITZ KB, PEARSON OH, ET AL. Current status of estrogen and progesterone receptors in breast cancer. Cancer 39:2934-2947, 1977.
12. EHRLICH MD, YOUNG PCM, and CLEARY RE. Progesterone receptors—a new approach to recurrent endometrial cancer. Proc Am Assoc Cancer Res 18:7, 1977.
13. LEGHA SS, DAVIS HL, and MUGGIA FM. Hormone therapy of breast cancer: new approaches and concepts. Ann Intern Med 88:69-77, 1978.
14. PAVLIK EJ, and COULSON PB. Modulation of estrogen receptors in four different target tissues: differential effects of estrogen vs progesterone. J Steroid Biochem 7:369-376, 1976.
15. JOLLES CJ, FREEDMAN RS, and JONES LA. Estrogen and progesterone therapy in advanced ovarian cancer: preliminary report. Gynecol Oncol 16:352-359, 1983.
16. HAMILTON TC, BEHRENS BC, LOUIE KG, ET AL. Induction of progesterone receptor with 17 beta-estradiol in human ovarian cancer. J Clin Endocrinol Metab 59:561-563, 1984.
17. WHO Handbook for Reporting Results of Cancer Treatment. Geneva, World Health Organization, 1979.
18. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.

Phase II Studies of Homoharringtonine in Patients With Advanced Malignant Melanoma; Sarcoma; and Head and Neck, Breast, and Colorectal Carcinomas[1,2]

Jaffer A. Ajani,* Isaiah Dimery, Sant P. Chawla, Kavitha Pinnamaneni, Robert S. Benjamin, Sewa S. Legha, and Irwin H. Krakoff[3]

Five phase II trials of the antitumor agent homoharringtonine were conducted in 80 patients who had advanced solid tumors. The five categories of solid tumors included malignant melanoma; sarcoma; and head and neck, breast, and colorectal carcinomas. The starting dose of homoharringtonine was 3.0–4.0 mg/m^2 by short iv infusion daily × 5 days every 21 days. Seventy-eight of 80 patients had had prior chemotherapy and 49 had had prior radiation therapy. Among the 74 evaluable patients in the five tumor categories, there were no complete or partial remissions. Homoharringtonine was generally well-tolerated. Nausea and vomiting, diarrhea, and fever and chills were the most common side effects. Serious reversible cardiovascular toxicity, which occurred in three patients, included symptomatic hypotension in two and short runs of ventricular tachycardia in one. The investigations thus conclude that homoharringtonine given by intermittent schedule is an inactive drug against these solid tumors previously exposed to chemotherapy. [Cancer Treat Rep 70:375–379, 1986]

Homoharringtonine is a cephalotaxine ester derived from the Chinese evergreen plant *Cephalotaxus harringtonia*. The compound was isolated by Powell et al in 1969 (1). The mechanism of action of homoharringtonine was defined by studies with L1210 leukemia and included evidence for inhibition of protein biosynthesis, DNA, and RNA (2). In preclinical studies, homoharringtonine was active against P388, Colon 38, $CD8F_1$ mammary carcinoma, L1210 leukemia, and B16 melanoma; it was inactive against Lewis lung carcinoma, human colon carcinoma, and lung and mammary xenografts (2). Schedule-dependent cytotoxicity against P388 murine tumor was also demonstrated; homoharringtonine was more active when given daily for 9 days than when it was administered infrequently (2). Its toxicity in animals included vomiting, myelosuppression, conduction abnormalities, and diarrhea. Human toxicity was defined in various phase I studies conducted in the United States and elsewhere (3–8). Hypotension and myelosuppression were the dose-limiting toxic effects. Hypotension was more pronounced when an iv bolus or short-infusion schedule was used (4–6). Myelosuppression was dose-limiting in studies using prolonged infusion of homoharringtonine (3,5). Nausea and vomiting, fever and chills, and alopecia were other side effects. Based on a phase I study of homoharringtonine (using intermittent short infusion) at the University of Texas M. D. Anderson Hospital, we began six phase II trials of this agent in patients who had advanced malignant melanoma; sarcoma; and head and neck, breast, ovarian, and colorectal carcinomas.

MATERIALS AND METHODS

A total of 80 patients with tumors from five categories were treated with homoharringtonine in various phase II trials. An additional phase II study of homoharringtonine in 17 patients who had advanced epithelial ovarian cancer was reported (9) and will be mentioned only briefly. In the 80 patients the tumor types included malignant melanoma; sarcoma; and head and neck, breast, and colorectal carcinomas. Patient characteristics are shown in table 1. Further details on prior therapy of patients in various tumor categories are shown in table 2. This would suggest that most of the patients had failed prior chemo-

[1]Received Apr 15, 1985; revised June 11, 1985; accepted June 18, 1985.

[2]Supported in part by contract N01-CM-27550 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medical Oncology (*J. A. Ajani, I. Dimery, S. P. Chawla, K. Pinnamaneni,* and *R. S. Benjamin*) and Chemotherapy Research (*S. S. Legha* and *I. H. Krakoff*), Division of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

* *Reprint requests to:* Jaffer A. Ajani, MD, Department of Medical Oncology, University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Box 68, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Characteristics of 80 patients entered in 5 phase II trials of homoharringtonine

Characteristic	No. of patients
Median age in yrs (range): 54 (16-77)	
Males/females	36/44
Tumor types	
Breast carcinoma	19
Colorectal carcinoma	17
Head and neck carcinoma	16
Sarcoma	16
Malignant melanoma	12
Performance status (Zubrod scale)*	
0	9
1	42
2	21
3	7
4	1
Prior therapy	
Chemotherapy	78
Radiation therapy	49
Surgery	79

* Median (range): 1 (0-4).

therapy. Eligibility criteria for entry in the study included a performance status ≤ 3 on the Zubrod scale; a life expectancy > 12 weeks; normal peripheral blood cell counts; normal serum creatinine and bilirubin and an absence of active cardiovascular disease levels; antitumor therapy during the preceding 4 weeks; and current antihypertensive therapy.

The starting dose of homoharringtonine was 3.0-4.0 mg/m^2 daily × 5. Patients who had a history of prior extensive chemotherapy (with mitomycin or nitrosoureas) or prolonged myelosuppression from prior chemotherapy or those who had received extensive radiation therapy were considered poor-risk patients and received an initial dose of 3.0 mg/m^2. The drug was administered in 1 L of normal saline over 4 hours, and an additional 1 L of normal saline was given to some patients before homoharringtonine administration. Patients were either hospitalized or treated in the outpatient chemotherapy areas. Careful monitoring of the patient's pulse and blood pressure was done on treatment days. If no significant toxicity was observed with the preceding course of treatment, the drug dose was escalated, not by exceeding the daily dose of 4 mg/m^2 but by increasing the treatment days from 5 to 6 or 7. Therapy was repeated every 21 days depending on the recovery from myelosuppression. If the preceding treatment course resulted in documented infection, bleeding due to thrombocytopenia, platelet count < 50,000/mm^3, or absolute granulocyte count < 500/mm^3, the dose of homoharringtonine was reduced by 25% for the subsequent course. Informed consent was obtained from each patient before initiation of therapy. Criteria for response were standard. Briefly, a complete remission was defined as the complete disappearance of all measurable disease for a minimum duration of 4 weeks; partial remission was defined as a ≥ 50% regression of measurable disease; minor response was defined as measurable regression of tumor but < 50% reduction from the initial size; no change was defined as no change in measurable tumor for a minimum duration of 6 weeks; and progressive disease was defined as the appearance of a new lesion(s) or measurable increase in the size of tumor(s).

RESULTS

A total of 142 courses of homoharringtonine were administered to 80 patients. The median number of courses given was one (range, one to four). The median dose of homoharringtonine was 4 mg/m^2 (range, 2.5-5). One patient received 5 mg/m^2 × 5 days as dose escalation instead of 4 mg/m^2 × 6 days; this constituted a minor protocol violation. Thirty-one percent of the patients received dose escalation and only 12.5% received dose reduction. The response to therapy of patients with tumors in the various categories is shown in table 3. Duration of stable disease or minor response did not exceed 12 weeks. The lack of complete or partial remission indicates that homoharringtonine was uniformly ineffective. Seventy-four of the 80 patients were evaluable for response to homoharringtonine. Six patients were inevaluable for the following reasons: one patient had a major protocol violation (his performance status was 4 on the Zubrod scale and he was receiving a pressor agent for profound hypotension due to infection when homoharringtonine therapy was initiated); one patient refused therapy on Day 2 of the second course when he redeveloped atrial fibrillation due to homoharringtonine; three patients were lost to follow-up after each received one course of homoharringtonine; and one patient refused therapy after the

TABLE 2.—Details of prior therapy of patients in various tumor categories

Tumor type	Chemotherapy		Radiation therapy (% of patients)
	% of patients	Median No. of regimens (range)	
Breast carcinoma	100	3 (1-9)	74
Colorectal carcinoma	100	1 (1-7)	29
Head and neck carcinoma	100	1 (1-2)	100
Sarcoma	100	2 (1-7)	62.5
Malignant melanoma	83	1 (0-3)	33

TABLE 3.—Response to homoharringtonine therapy

Tumor type	No. of evaluable patients	Minor response	No change	Progressive disease
Ovarian carcinoma*	17	0	0	17
Sarcoma	16	1	0	15
Head and neck carcinoma	16	0	1	15
Colorectal carcinoma	15	0	1	14
Breast carcinoma	15	0	1	14
Malignant melanoma	12	1	1	10
Total	91	2	4	85

*See ref 9.

first course and could not be evaluated for response.

If it is assumed that homoharringtonine has a true response rate of 20%, one or more responses would have been observed among 12 patients with malignant melanoma, with a probability > 90%. Among patients with other tumor types there would have been 95% probability of observing one or more responses (10).

Toxicity

The hematologic and nonhematologic toxic effects observed in the 80 patients are shown in tables 4 and 5. Nonhematologic toxic effects were graded based on the recommendations made by the World Health Organization (11). We believe that adequate doses of homoharringtonine were given, resulting in the desired biologic effect on the bone marrow. Myelosuppression was further characterized in patients with individual tumor types as shown in table 6. Thirty courses (21%) resulted in a lowest platelet count < 50,000/mm^3, and 29 courses (20%) resulted in a lowest granulocyte count < 1000/mm^3. Generally, homoharringtonine therapy was well-tolerated. Nausea and vomiting, diarrhea, and fever and chills were the other common side effects. Diarrhea usually occurred after Day 10 and was resolved by Day 18. It was never severe enough to require hospitalization to replenish fluids. Fever and chills were well-controlled by premedication with acetaminophen and diphenhydramine hydrochloride. Nausea and vomiting was generally mild and well-controlled with antiemetic therapy. Two patients received fluids iv to correct the volume loss caused by vomiting. Symptomatic hypotension (systolic pressure ⩽ 70 mm Hg) was observed in two patients only at doses of 3 and 4 mg/m^2 and was corrected using fluids along with immediate discontinuation of homoharringtonine. Neutropenic fever in eight patients was cured with broad-spectrum antibiotics given in the hospital. Other minor toxic effects included anorexia, weakness, transient macular erythematous rash, and wheezing. A spectrum of cardiac abnormalities observed in eight patients included the following: reversible atrial fibrillation on initiation of homoharringtonine twice in one patient, sinus tachycardia in four, premature atrial contractions of no clinical consequence in one, premature ventricular contractions of no clinical consequence in one, and short runs of ventricular tachycardia during the first and second courses of homoharringtonine (4 mg/m^2 each) in a 24-year-old woman. Nevertheless, all arrhythmias resolved following completion of the therapy. An anemic 77-year-old man had congestive heart failure 2 weeks after his second course of homoharringtonine (each course, 3 mg/m^2). His heart failure responded readily to conventional therapy; contribution of homoharringtonine to the development of the heart failure is uncertain.

DISCUSSION

Seventy-eight of 80 patients studied in this report had received prior chemotherapy, and 49 had had prior radiation therapy. Homoharringtonine was generally well-tolerated and was given at adequate dose levels to induce a biologic effect; however, no complete or partial response was observed. We acknowledge that we treated only 12 evaluable patients with malignant melanoma which results in a 90% probability of not missing a ⩾ 20% response to homoharringtonine (10). We treated at least 14 evaluable patients with breast carcinoma, sarcoma, colorectal carcinoma, and head and neck carcinoma, raising this probability to 95%. Clinical studies published in the Chinese literature (12) and English literature (2) had inspired hope that homoharringtonine might be an active drug in the treatment of patients with solid tumors or

TABLE 4.—Hematologic toxicity of homoharringtonine therapy

	Lowest median*	Range*	Lowest median day (range)
Wbc count	2.6	< 0.1–12.4	15 (3–60)
Absolute granulocyte count	1.4	0–11.4	15 (3–60)
Platelet count	88	4–453	12 (4–60)

* × 10^3 cells/mm^3.

TABLE 5.—Nonhematologic toxicity of homoharringtonine therapy

Toxic effect	No. of patients	Toxicity grade* 1	2	3	4
Nausea and vomiting	40	23	17	0	0
Fever and chills	29	10	27	0	0
Diarrhea	26	0	21	5	0
Neutropenic fever	8	0	0	8	0
Cardiac	8	5	2	0	1
Rash	5	5	0	0	0
Anorexia	4†				
Weakness	4†				
Hypotension	2†				
Alopecia	2	2	0	0	0

*See ref 10.

†No provision for grading in the toxicity criteria of the World Health Organization; all were mild, except hypotension, which was symptomatic and reversible.

TABLE 6.—Hematologic toxicity of homoharringtonine as observed in various tumor types*

	Wbc count ($\times 10^3$/mm^3)		Absolute granulocyte count ($\times 10^3$/mm^3)		Platelet count ($\times 10^3$/mm^3)	
	< 4.0 but > 3.0	⩽ 3.0	< 2.0 but > 1.0	⩽ 1.0	⩽ 100 but > 50	⩽ 50
Colorectal carcinoma						
Patients	47	50.5	35	41	29	53
Courses	30	48	22	30	18.5	37
Breast carcinoma						
Patients	26	63	26	42	21	42
Courses	21	55	17	38	17	31
Head and neck carcinoma						
Patients	19	75	25	50	37.5	31
Courses	11	67	18.5	37	26	22
Sarcoma						
Patients	25	47	31	44	31	31
Courses	21	55	21	37	27.5	21
Malignant melanoma						
Patients	8	67	8	42	42	25
Courses	4.5	55	4.5	41	23	18

*Values = %.

leukemia. Our results, however, are disappointing. The dose-limiting hypotension previously observed with intermittent schedules (4-6) was not a problem in these six phase II studies in 97 patients. Reduced incidence of symptomatic hypotension is attributed to the limiting of the daily homoharringtonine dose to 4 mg/m^2. Although some interest has been expressed in altering the homoharringtonine schedule to one of continuous infusion for up to 30 days, based on the disappointing results reported herein, we have no plans to further explore this drug in the patients with solid tumors. In conclusion, homoharringtonine given by intermittent schedule is an inactive drug against these previously treated solid tumors.

REFERENCES

1. POWELL RG, WEISLEDER D, SMITH CR, JR, ET AL. Structure of cephalotaxine and related alkaloids. Tetrahedron Lett 46:4081-4084, 1969.
2. Clinical Brochure. Homoharringtonine (NSC-141633). Bethesda, MD, National Cancer Institute, Aug 1982.
3. COONLEY CJ, WARRELL RP, JR, and YOUNG CW. Phase I trial of homoharringtonine administered as a 5-day continuous infusion. Cancer Treat Rep 67:693-696, 1983.
4. LEGHA SS, KEATING M, PICKETT S, ET AL. Phase I clinical investigation of homoharringtonine. Cancer Treat Rep 68:1085-1091, 1984.
5. MALAMUD SC, OHNUMA T, COFFEY V, ET AL. Phase I study of homoharringtonine (HHT) in 10 day schedule: 6 hour infusion daily vs continuous infusion. Proc Am Assoc Cancer Res 25:179, 1984.
6. WHITACRE MY, VAN ECHO DA, APPLEFELD M, ET AL. Phase I study of homoharringtonine (NSC 141-633). Proc ASCO 2:C-129, 1983.

7. HACKER MP, STEWART JA, NEWMAN RA, ET AL. Toxicologic studies of homoharringtonine in humans and rats. Proc Am Assoc Cancer Res 23:1287, 1983.
8. MAYERNIK D, STEVEN CK, and NEIDHART J. Acute cardiac arrhythmias with phase I and II agents. Proc ASCO 1:C-53, 1982.
9. KAVANAGH JJ, GERSHENSON DM, COPELAND LJ, ET AL. Intermittent homoharringtonine for the treatment of refractory epithelial carcinoma of the ovary: a phase II trial. Cancer Treat Rep 68:1503-1504, 1984.
10. GEHAN EA, and SCHNEIDERMAN MA. Experimental design of clinical trials. *In* Cancer Medicine (Holland J, and Frei E, eds). Philadelphia, Lea and Febiger, 1978, pp 503-505.
11. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207-214, 1981.
12. Cephalotaxus Research Coordinating Group. Cephalotaxine esters in the treatment of acute leukemia: a preliminary clinical assessment. Chin Med J 2:263-272, 1976.

Effect of Tamoxifen on Immune Functions[1]

Heikki Joensuu,* Auli Toivanen, and Eeva Nordman[2]

Tamoxifen (20 mg twice daily) was given to ten patients with breast cancer whose immune functions were determined prior to and 3, 6, and 12 months after starting tamoxifen therapy. No consistent changes could be observed in erythrocyte rosette-forming cell counts, erythrocyte antibody rosette-forming cells, active erythrocyte rosette-forming cells, theophylline-resistant rosette-forming cells, surface immunoglobulin-positive cells, and responses to mitogens phytohemagglutinin and concanavalin A. Erythrocyte antibody rosette-forming cells decreased significantly, however, at 6 months and active erythrocyte rosette-forming cells at 12 months after starting tamoxifen. We conclude that no change in the immune capacity could be detected during tamoxifen treatment. [Cancer Treat Rep 70:381–382, 1986]

Tamoxifen is an antiestrogenic drug that has been widely used in the adjuvant treatment of locally advanced breast cancer and in the management of metastatic disease. Compared to cytostatic therapy and many other hormones used in the treatment of breast cancer, tamoxifen has few side effects, among which hot flushes, vaginal bleeding, initial hypercalcemia, tumor pain, fluid retention, skin rashes, and occasional gastric symptoms are the most important but only rarely seen by the clinician.

The effect of tamoxifen on immune functions and host resistance has not, however, been thoroughly studied and world literature is sparse. Webster et al (1) reported no detectable effect on the immune response during tamoxifen treatment in patients with breast cancer. Paterson et al (2) found a significant rise in mean T-cell counts in the beginning of tamoxifen treatment in responders and falling T-cell counts before other evidence of disease progression. They concluded that T-cell counts may permit predictive evaluation of the response during tamoxifen treatment. Because tamoxifen is so commonly used and given to cancer patients even for several years, we decided to study its possible effects on the immune capacity.

MATERIALS AND METHODS

Tamoxifen was given at a dose of 20 mg twice daily orally to ten histologically diagnosed patients with breast cancer. The mean age of the patients was 63.3 years, and two of them were receiving the drug on an adjuvant basis. In all patients surgery had been performed on the primary tumor and in nine, postoperative or other radiotherapy had been given. No patient had received cytostatic therapy. If the treatment had to be changed due to disease progression, the immunological follow-up was discontinued. One patient achieved complete remission, one achieved partial remission, three had stable disease, and four had disease progression. In one patient, the treatment response could not be evaluated. Erythrocyte rosette cell counts (mainly T cells) were determined according to Jondal et al (3). The number of suppressor cells was estimated using the ability of theophylline to inhibit E-rosette formation of human lymphocytes (4,5). Surface immunoglobulin-positive cells (number of B lymphocytes) were determined as described earlier (6). The proliferative response of lymphocytes to phytohemagglutinin and concanavalin A was determined by the micromethod described by Eskola et al (7). Erythrocyte antibody rosette-forming cells (cells having receptors for the Fc-region) were determined using washed sheep erythrocytes (SRBC) coated with subagglutinating amounts of chicken anti-SRBC serum as described earlier (8). The active erythrocyte rosette-forming cells, which may represent a subpopulation of T lymphocytes that function primarily as effector lymphocytes, were determined by counting the number of SRBC rosettes after centrifuging 250 μl of SRBCs with 250 μl of patient lymphocytes for 5 minutes at 1000 rpm. These immunological tests were done before starting tamoxifen (Sample I) and at 3 (Sample II), 6 (Sample III), and 12 (Sample IV) months after beginning the drug. The statistical significance of the

[1]Received Feb 21, 1985; revised June 3, 1985; accepted June 14, 1985.

[2]Departments of Radiotherapy (*H. Joensuu* and *E. Nordman*) and Medical Microbiology and Medicine (*A. Toivanen*), University Central Hospital of Turku, Finland.

**Reprint requests to:* Heikki Joensuu, MD, Department of Radiotherapy, University Central Hospital of Turku, SF-20520 Turku, Finland.

TABLE 1.—Effect of tamoxifen on 7 immunological parameters

Sample	E%	EA%	Eact%	th-res%	S-Ig%	Phyto-hemagglutinin*	Concanavalin A*
I	67.9	10.5	45.5	34.3	7.9	36,600	22,700
II	59.4	11.6	35.8	19.5	9.0	38,500	19,400
III	64.9	2.7†	35.7	17.4	7.9	40,700	29,800
IV	51.0	3.8	16.8‡	28.2	6.5	44,900	18,800

*Net counts/min.
†$P < 0.05$ when compared to Sample I.
‡$P < 0.02$ when compared to Sample I.

changes in the immunological parameters when compared with the original values was determined with Student's t test.

RESULTS

The results are shown in table 1. No statistical changes could be observed in erythrocyte rosette-forming cell counts (E%), theophylline-resistant rosette counts (th-res%), surface immunoglobulin-positive cells (S-Ig%), or responses to mitogens phytohemagglutinin or concanavalin A. Erythrocyte antibody rosette-forming cells (EA%) decreased from 10.5 to 2.7 at 6 months after starting tamoxifen ($P < 0.05$) and active erythrocyte rosette-forming cells (Eact%) decreased from 45.5% to 16.8% at 12 months after starting tamoxifen ($P < 0.02$). None of the parameters could be used in predicting treatment response to tamoxifen. No consistent difference in the immunological tests performed could be seen between clinically responding (one complete response; one partial response) and nonresponding patients.

DISCUSSION

The SRBC rosette-forming cell counts and surface immunoglobulin-positive cell counts remained stable throughout the study, indicating that there was no significant change in the relative number of T and B lymphocytes. However, erythrocyte antibody rosette-forming cell counts decreased significantly 6 months after starting tamoxifen, indicating decrease in cells having Fc receptors on their surface. Also, active SRBC rosette-forming cells decreased after 12 months of tamoxifen treatment. Because of the late appearance of these changes, clinically undetectable progression of cancer may have influenced the results. These findings should be confirmed in a larger study. The proliferative response of lymphocytes to mitogens phytohemagglutinin and concanavalin A remained unchanged, so there was no change in the functional capacity of lymphocytes in this respect.

Because tamoxifen is at present and has been for many years widely used in breast cancer, it is essential to know whether it has any long-term effects on the immune capacity of cancer patients. To the best of our knowledge this is the first study in which several immunological parameters have been followed for up to 1 year during tamoxifen treatment. Our results largely confirm the finding of Webster et al (1) that tamoxifen does not cause any significant immune suppression in patients with breast cancer and it is thus superior to chemotherapy and radiotherapy, which both are immunosuppressive (9). It is improbable that tamoxifen would cause significant additional immunosuppression when used concomitantly with chemotherapy or radiotherapy.

REFERENCES

1. WEBSTER DJT, RICHARDSON G, BAUM M, ET AL. Effect of treatment on the immunological status of women with advanced breast cancer. Br J Cancer 39:676, 1979.
2. PATERSON AG, and WEBSTER DJT. The predictive value of T-lymphocyte counts in advanced breast-cancer patients treated with tamoxifen. Br J Cancer 54:637, 1982.
3. JONDAL M, HOLM G, and WIGZELL H. Surface markers of human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. J Exp Med 136:207, 1972.
4. LIMATIBUL S, SHORE A, DOSCH HM, ET AL. Theophylline modulation of E rosette formation: an indicator of T cell maturation. Clin Exp Immunol 33:503, 1978.
5. SHORE A, DOSCH HM, and GELFAND EW. Induction and separation of antigen-dependent T helper and T suppressor cells in man. Nature 274:586, 1978.
6. PETTERSSON D, MELLSTEDT H, and HOLM G. IgG on human blood lymphocytes studied by immunofluorescence. Scand J Immunol 8:535, 1978.
7. ESKOLA J, SOPPI E, VILJANEN M, ET AL. A new micromethod for lymphocyte stimulation using whole blood. Immunol Commun 4:297, 1975.
8. NOWAK JS, LASSILA O, VAINIO O, ET AL. IgG Fc receptor-bearing cells during early lymphoid cell development in the chicken. Cell Immunol 74:198, 1982.
9. TOIVANEN A, and NORDMAN E. Long-term effect of postoperative irradiation on the immune functions in patients with mammary carcinoma. Acta Radiol Oncol 20:119, 1981.

Pentamethylmelamine: Review of an Aqueous Analog of Hexamethylmelamine[1]

Brenda J. Foster,* Kathleen Clagett-Carr, Daniel Hoth, and Brian Leyland-Jones[2,3]

The *s*-triazine derivatives have shown preclinical antitumor activity against several histologic types. The most widely used compound of this class in the clinic, hexamethylmelamine, has been largely restricted to oral use because of its low solubility and lack of stability in solutions suitable for parenteral administration. New analogs were sought which were soluble and stable and retained antitumor activity. Pentamethylmelamine (PMM), the monodemethylated derivative, showed these promising characteristics. Preclinical toxicology studies of PMM in mice, dogs, and monkeys showed toxic manifestations that involved the hematopoietic, lymphatic, renal, male reproductive, gastrointestinal, and nervous systems; these changes were both infusion-rate- and dose-dependent. Clinical phase I trials of PMM were performed using a variety of infusion durations and frequency schedules. The dose-limiting toxic effect common to all of these trials was protracted nausea and vomiting. In addition, some studies reported dose-limiting central nervous system manifestations in the form of agitation, drowsiness, somnolence, and even coma. Mild to moderate hematologic changes were noted. Because of the severity and frequency of the gastrointestinal and central nervous system toxic effects observed in the completed trials, no new clinical trials of PMM sponsored by the National Cancer Institute are planned. However, the interest in finding a clinically useful parenteral triazine continues. [Cancer Treat Rep 70:383–389, 1986]

Early clinical trials with hexamethylmelamine (HMM) demonstrated activity against human lymphomas, ovarian carcinoma, and small cell lung carcinoma (1,2). A major limitation to its clinical usefulness has been its low aqueous solubility; consequently, it is only administered by the oral route. However, it can cause pronounced gastrointestinal manifestations of nausea and vomiting, abdominal cramps, and diarrhea, which negatively influence patient compliance with oral medication. In addition, studies have demonstrated that the bioavailability of HMM when administered by the gastrointestinal route is highly variable (3). Because the use of this clinically active drug is limited by problems of gastrointestinal toxicity coupled with variable bioavailability and patient compliance, the idea of a parenteral form of HMM or a water-soluble analog is very attractive.

Original attempts to synthesize a parenteral alternative used soluble products from the complexation of HMM with gentisic acid (4). These complexes were unstable at physiologic pH. This lack of stability, in addition to local irritation from the gentisate, precluded their clinical usefulness. Water-soluble analogs of HMM were studied at the Chester Beatty Research Institute, London, and a series of demethylated derivatives were examined (5). Of these demethylated products, pentamethylmelamine (PMM), the monodemethylated derivative, was approximately 24 times more water soluble than HMM and showed therapeutic effectiveness against a lung tumor xenograft (P246) nearly equivalent to that of HMM.

BIOCHEMISTRY

Metabolism

The chemical structural similarities of HMM and PMM can be seen in figure 1. Analytical studies were performed by Worzalla et al (6,7) in humans and rodents following the oral or ip administration of either HMM-methyl ^{14}C or HMM-ring ^{14}C. Results from the HMM-methyl ^{14}C studies demonstrated the appearance of respiratory ^{14}C within 1 hour (6). Gas chromatographic and mass spectrophotometric analysis of the urine identified four major metabolites. There were no species differences

[1]Received Apr 19, 1985; accepted May 31, 1985.

[2]Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]We thank Mrs. Michelle Smith for her assistance in the preparation of this manuscript.

*Reprint requests to: Dr. Brenda J. Foster, Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Landow Bldg, Rm 4C09, National Institutes of Health, Bethesda, MD 20892.

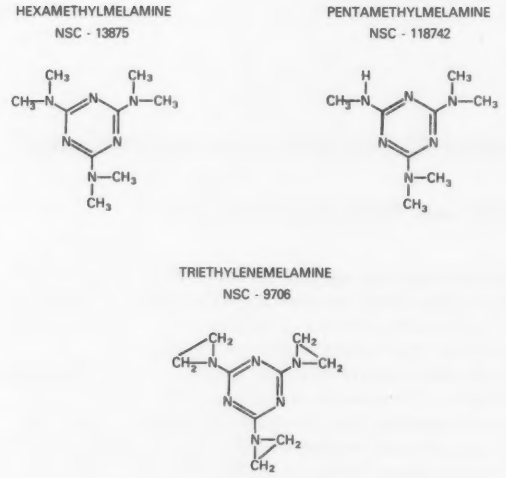

FIGURE 1.—Structures of HMM, PMM, and triethylenemelamine.

in the metabolites detected. The major metabolites were identified as N^2,N^4,N^6-trimethylmelamine, N^2,N^4-dimethylmelamine, monomethylmelamine, and melamine (6,7). No unmetabolized HMM could be identified in the urine at 72 hours. It was demonstrated, however, that the *s*-triazine ring was extremely stable and that there was no significant metabolic cleavage (7). They postulated the sequence of *N*-demethylation shown in figure 2 as the metabolic pathway. Although there are two possible structural isomers of tetramethylmelamine, trimethylmelamine and dimethylmelamine, they were able to identify only one (solid arrows). Thus, a dimethylamino group appeared to be demethylated preferentially to a monomethylamino group (6,7). Metabolism of HMM and PMM is mediated by mixed function oxidases and there is good evidence that this involves the formation of intermediates (*N*-methylolamine derivatives) and formaldehyde as shown in figure 3 (8,9).

Structure-Cytotoxicity Relationships

There is extensive evidence that both the presence of methyl groups and metabolic activation of HMM and PMM are necessary for cytotoxic activity (8-13). During the activation process, methylolmelamine intermediates are formed along with formaldehyde. There are conflicting results, depending on the tumor model used and how cytotoxic activity is measured, on whether the methylol intermediate or the formaldehyde generated is the major species responsible for the cytotoxic effect (8-13). Even in studies where a binding agent is used for the generated formaldehyde (eg, semicarbazide), the results are conflicting (8,9,11,13).

There appears to be some correlation between the degree of demethylation and antitumor activity. The total absence of methyl groups (ie, melamine) results in an inactive compound (10). The ability of the methylmelamines to undergo demethylation decreases with the number of methyl groups in the molecule (8). Lake et al (10) investigated the toxicity and antitumor effects of HMM and nine *N*-demethylated compounds in $BD2F_1$ mice bearing either Sarcoma 180 or Lewis lung carcinoma. From their studies the decrease in potency was gradual when the number of methyl groups was reduced from six to four. However, when the methyl groups were reduced to two or less there was a precipitous decrease in potency. An effect of structural change on activity was shown for the homologs that had their positional isomers included in the studies (eg, N^2,N^2,N^4,N^4-tetramethylmelamine and N^2,N^2,N^4,N^6-tetramethylmelamine; N^2,N^2-dimethylmelamine; and N^2,N^4-dimethylmelamine). The

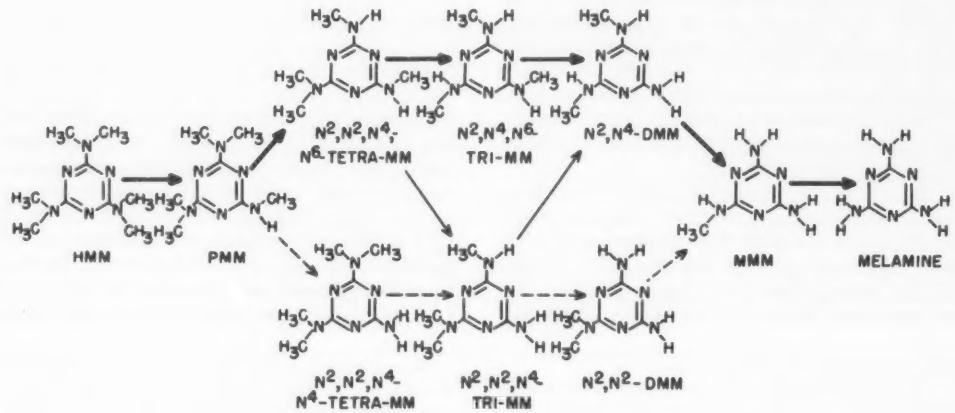

FIGURE 2.—Suggested sequence of *N*-demethylation. Printed by permission from Ref 6.

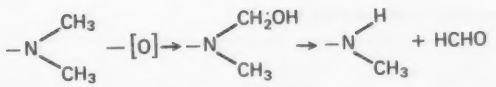

FIGURE 3.—Formation of *N*-methylolamine derivatives and formaldehyde. Printed by permission from Ref 8.

most toxic and therapeutic positional isomérs were those that possessed the most dimethyl-substituted nitrogens (10).

Mechanism of Action

The exact mechanism of HMM and PMM cytotoxicity is unknown. Both bear structural similarities (fig 1) to triethylenemelamine, a classic alkylating agent (1,14), although they do not show consistent collateral sensitivity with other classic alkylating agents in rodent tumor models (15). The categories of human malignancies that have been responsive to HMM are the same as those showing responsiveness to alkylating agents (1,16). The latter may be a function of the responsiveness of the histologic tumor type rather than an indication of the intracellular target of cytotoxic effect. Neither HMM nor PMM demonstrated significant alkylating activity with the in vitro 4-(*p*-nitrobenzyl)pyridine assay (6). Thus, it is difficult to determine from the results available whether or not these agents are alkylating agents.

Because some triazine compounds can act as folic acid antagonists (17), in vitro experiments using dihydrofolate reductase isolated from rat liver revealed no dihydrofolate reductase inhibition by HMM or its metabolites (7). This makes folic acid antagonism an unlikely cytotoxic effect for these compounds.

Pharmacokinetics

Pharmacology studies have been performed in mice, rats, rabbits, and humans (3,6,7,18–27).

Comparative studies in mice with HMM and PMM given ip revealed a longer half-life for HMM (44.5–49 minutes) than for PMM (7.6–8.7 minutes) when both drugs were given at the same dose (50 and 100 mg/kg) (18). Similar results were reported in rabbits (19). The corresponding area under the concentration-versus-time curve was also higher for HMM. Plasma protein binding was 74% and 71% for HMM and PMM, respectively (18). Other comparative studies in mice after administration of ring-^{14}C-labeled drug at one-tenth of the lethal dose for each compound (175 mg/kg for PMM and 200 mg/kg for HMM) showed a similar plasma disappearance (approximately 40 minutes) for both (20). The liver and small intestines contained the highest tissue concentrations of the radiolabel. The maximum brain concentration of PMM was 14-fold higher than that obtained with HMM (20). The rate of PMM elimination was dose-dependent in the rat, with a lower dose giving a higher plasma clearance value, ie, the lower dose showed a shorter half-life (21).

Species comparisons of the rate of *N*-demethylation of PMM revealed the metabolism to be more rapid in mice than in rats and slower in man than in either rat or mouse (22). Cytotoxic levels of *N*-methylolmelamine, a suspected active intermediate, were detectable in mice and rats treated with 90 mg/kg. In patients who had been treated with PMM at doses of 100–1300 mg/m^2, these compounds were not detectable (22).

In most patients the plasma elimination of PMM best fit a two-compartment open model. The peak plasma level was dose-dependent, with the terminal half-life of the parent compound reported to be approximately 135 minutes (19,23,24). Less than 0.1% of the administered dose of PMM was excreted in the urine unchanged within the first 24 hours (23,24). Casper et al (25) studied PMM pharmacokinetics in patients after administration of ^{14}C ring-radiolabeled drug and found a postinfusion half-life of 12 hours for the radiolabel, with approximately 70% of the total radiolabel being excreted in the urine.

Since the elimination of PMM from the central compartment is largely dependent on metabolism by demethylation, it is not surprising that a prolonged terminal half-life of the parent drug of up to > 6 hours occurred in patients with abnormal liver function (26,27). Benvenuto et al (26) found the plasma clearance of the demethylated metabolites to also be related to hepatic function, with decreased clearance in patients with abnormal hepatic function. In addition, central nervous system toxic effects were found to be directly proportional to the severity of hepatic impairment (26).

PRECLINICAL ACTIVITY

In animal tumor models, PMM had activity similar to HMM as shown in table 1. It was active against the P246 tumor, a human bronchial carcinoma which is grown in immune-deprived mice (thymectomized and irradiated) (5); Sarcoma 180 (10), Lewis lung carcinoma (10), and M5076 ovarian carcinoma (28). In the subrenal capsule assay, PMM showed activity against the MX-1 and MX-2 breast tumor xenografts (28).

Schedule-dependency studies using daily and intermittent treatment schedules were conducted in the murine M5076 ovarian carcinoma and in the Lieberman plasma cell No. 1 (LPC-1) models. The best activity was seen with daily treatment on Days 1–17. No activity was seen when PMM was given as a single bolus or as a fractionated dose over Day 1. Activity was reported using oral, ip, and sc drug administration. Daily oral treatment was slightly more effective than daily treatment with the ip or sc routes. The therapeutic index was narrow, with activity demonstrated only at or near the toxic dose level on most schedules and little or no activity at lower dose levels (28).

TABLE 1.—Preclinical antitumor activity of PMM and HMM*

Tumor (site)	Drug: Schedule	Dose/injection (mg/kg)	Route	Antitumor parameter	PMM	HMM	Ref No.
P246 (ip)	Daily × 5; 9-day rest; daily × 2	90	ip	Change in tumor volume with treatment	—	0	5
	Daily × 5; 9-day rest; daily × 2	60	ip			15%	
	Daily × 5; 9-day rest; daily × 5	90	ip		0.1%	—	
	Daily × 5; 9-day rest; daily × 5	60	ip		9.0%	—	
Sarcoma 180 (sc)	Daily × 5	96	ip	% inhibition of tumor growth	—	82%	10
	Daily × 5	108	ip		75%		
Lewis lung (sc)	Daily × 5	96	ip	% inhibition of tumor growth	—	80%	10
	Daily × 5	108	ip		88%		
M5076 ovarian carcinoma (sc)	Daily × 9	60	ip	Tumor weight inhibition	73%	—	28
MX-1 (src)†	Daily × 10	60	sc	Tumor weight inhibition	++*	—	28
MX-2 (src)†	Daily × 10	60	sc	Tumor weight inhibition	++*	—	28

* Animals that were treated had regression of tumor as compared to tumor size at start of treatment.
† src = subrenal capsule xenograph.

PRECLINICAL TOXICOLOGY

Acute animal toxicity studies were performed in CDF_1 mice, beagle dogs, and rhesus monkeys (28). Mice were dosed using a single ip injection. Dogs and monkeys were injected iv either once, once daily for 5 days, or once daily for 10 days. The defined doses by species are shown in tables 2 and 3 along with the LD50 for HMM. PMM was administered as a solution of 50 or 100 mg/ml in sterile saline at pH 3.0.

In mice, histologic changes were detected in the cardiovascular, reproductive, and respiratory systems. Over 50% of the changes showed some form of myocardial degeneration, mineralization, or atrial thrombosis. Determination of the reversibility of these lesions could not be meaningfully assessed, since the longest observation period in mice was 14 days posttreatment. Reproductive system changes were seen only in males and consisted of moderate testicular degeneration. Approximately 20% of the mice had lung changes which consisted of pulmonary congestion or hemorrhage.

In dogs and monkeys, the clinical manifestations of drug toxicity occurred primarily during or immediately following drug administration. The dramatic signs, ie, convulsions, were present at most dose levels and were infusion-rate-dependent. In addition, lethality could be avoided by administering several smaller doses spaced 1 hour apart. Other manifestations seen in both dogs and monkeys included anorexia, weight loss, salivation, adipsia, emesis, diarrhea, hyperactivity or hyperexcitability, tetanic seizures, depression, weakness, dyspnea, and skin changes at the site of injection. Only dogs showed incontinence of urine and/or stool, ataxia, paresis, somnolence, and lethargy. Toxic manifestations seen only in monkeys were anuria and acoprosis.

Laboratory studies revealed mild hematologic and clinical chemistry alterations. These were generally transient, especially at sublethal doses. Anemia; reticulocytopenia; lymphopenia; thrombocytopenia; monocytosis; increased levels of BUN, SGOT, SGPT, and creatinine; and hypoglycemia were observed in dogs and monkeys, primarily at lethal dose levels. Additionally, monkeys displayed reduced alkaline phosphatase levels, while this enzyme was elevated in dogs.

Histologically, consistent changes seen in dogs and monkeys involved the lymphoid, gastrointestinal, renal, male reproductive, integumentary (injection site), and hematopoietic systems (table 4). Inconsistent changes that did not occur in all animals were noted in the liver and heart. The changes seen in the respiratory system were felt to be primarily the result of unconsciousness at the time of seizures. No neuropathology was noted in either the dogs or monkeys.

TABLE 2.—Lethal doses of PMM for CDF_1 mice after single ip injection (combined sexes)*,†

	Dose: mg/kg	Dose: mg/m^2
LD90	303	910
LD50	232	698
LD10	178	535

* *From* Ref 29.
†Dose of HMM that caused lethality in 50% of mice when given as a single ip dose was 220 mg/kg (660 mg/m^2).

CLINICAL TRIALS

PMM was evaluated in eight phase I trials using seven different schedules (table 5) (24,25,27,29–33). Two hundred nineteen patients with nonhematologic solid tumors were evaluable in these trials. The dose-limiting toxic ef-

TABLE 3.—Lethal and toxic doses of PMM for dogs and monkeys*

No. of injections	Lethality and toxicity†	Dog dose		Monkey dose	
		mg/kg	mg/m²	mg/kg	mg/m²
1	LD	200.0	4000.0	—	—
	TDH	100.0	2000.0	—	—
	TDL	12.5	250.0	—	—
	HNTD	6.25	125.0	—	—
5	LD	100.0	2000.0	166.7	2000.0
	TDH	50.0	1000.0	83.3	1000.0
	TDL	12.5	250.0	20.83	250.0
	HNTD	6.25	125.0	10.42	125.0
10	LD	50.0	1000.0	—	—
	TDH	25.0	500.0	—	—
	TDL	6.25	125.0	—	—
	HNTD	3.13	62.5	—	—

* *From* Ref 29.
†LD = lethal dose; TDH = toxic dose high; TDL = toxic dose low; and HNTD = highest nontoxic dose.

fect in all studies was severe nausea and vomiting uncontrolled by antiemetics. Neurotoxicity (peripheral and central) in the form of weakness, ataxia, slurred speech, confusion, and coma was also dose-limiting on the single (29,30), daily × 5, and daily × 10 (31,32) schedules. While neurotoxicity was mild at the lower dose levels, it became more serious and consistent at dose levels > 1200 mg/m² on the single, daily × 5, and daily × 10 schedules (29–32). Although neurotoxicity was also seen on the weekly 24-hour infusion schedule at Memorial (25), the weekly 1-hour infusion schedule at the Washington, DC, Veterans Hospital (24), and the intermittent Day-1, -3, and -5 schedule at Baltimore (30), it was not found to be dose-limiting. The toxic effects combination of severe nausea and vomiting and decreased cortical function made the administration of PMM difficult.

Myelosuppression was mild on most schedules, but moderate to severe thrombocytopenia was seen on the daily × 5 (31) and daily × 10 (32) schedules. Mild hepatic dysfunction (24,29) and skin rash (25,27) were also reported, although the latter was not definitely attributable to PMM.

Besides one partial response reported in a patient with renal carcinoma (25), no other major responses were seen in any of the phase I studies. Nine minor responses were reported: one each in melanoma (31), hepatoma (31), squamous cell carcinoma of the bronchus (27), small cell lung carcinoma (24), carcinoma of the colon (24), pleural

TABLE 4.—Histologic changes in large animals*

System	Dog	Monkey
Lymphatic	Lymphoid depletion; edema and congestion; hemorrhage; hemosiderosis; erythrophagocytosis; necrosis; inflammation; plasmacytosis; plasma cell necrosis	Same as in dog
Gastrointestinal	Congestion; villus atrophy; lacteal dilation; ulceration; degeneration; fibroplasia	Same as in dog
Hepatic	Centrilobular necrosis; congestion; degeneration; hypertrophy of central veins (dogs only)	Same as in dog
Renal	Crystalline deposition; glomerular dilation and atrophy	Tubular pigmentation; medullary congestion
Cardiovascular	Atrial hemorrhage and necrosis; focal ventricular myocardial inflammation and degeneration	Ventricular hemorrhage
Reproductive (male)	Testicular degeneration	No changes
Respiratory	Emphysema; inflammation; edema and congestion; necrosis and fibrosis	No changes
Integumentary	Edema; inflammation; hemorrhage; thrombosis; degeneration; necrosis and fibrosis	Same as in dog
Hematopoietic	Bone marrow depletion	Same as in dog
Neurologic	No changes	No changes

* *From* Ref 29.

TABLE 5.—Phase I PMM studies

Schedule	Dose (mg/m²) Range	Maximum tolerated dose	No. of evaluable patients	Toxicity Dose-limiting	Toxicity Other	Ref No.
1-hr weekly infusion	80-1500	1000	34	Nausea and vomiting	CNS (somnolence, drowsiness, paresthesia); elevated liver function tests; mild myelosuppression	24
24-hr continuous infusion weekly	50-3000	2000	26	Nausea and vomiting	CNS (agitation)	25
1-4-hr infusion	100	1300	17	Nausea and vomiting	Somnolence	27
Single dose every 4 wks	40-2400	1200-1800	22	Nausea and vomiting; neurotoxicity	CNS (drowsiness, agitation, confusion, coma); elevated liver function tests; serum creatinine	29
2-hr iv single dose every 3 wks	80-2000	1500	30	Nausea and vomiting; CNS	Depression; headache; confusion	30
Single dose		1000	5	Nausea and vomiting		
2-hr infusion daily × 5 every 3-4 wks	80-2500	2000	42	Nausea and vomiting; neurotoxicity	CNS (reversible coma, slurred speech, amnesia, hearing loss, somnolence); myelosuppression	31
2-6-hr infusion daily × 10 every 3 wks	40-1200	750-1000	16	Nausea and vomiting; neurotoxicity	Thrombocytopenia; confusion	32
Weekly × 4 every 6 wks	80-1000	560	27	Nausea and vomiting; neurotoxicity		33

mesothelioma (30), carcinoma of the parotid gland (30), and breast and ovarian carcinomas (31).

Due to the toxic effects encountered in phase I trials, only one phase II study was activated. This was a randomized study performed in Egypt in patients with bilharzial bladder cancer (34) comparing HMM to PMM. A response rate of 35% (seven partial responses among 20 evaluable patients) was reported for the HMM-treated arm and 32% (one complete response and seven partial responses among 25 evaluable patients) was reported for the PMM-treated arm. Toxic effects were reported as equivalent for both arms.

CONCLUSION

The *s*-triazines represent a unique class of compounds with antitumor activity (1,2,5,10,11,28). HMM is the most widely used agent from this class, but largely because of the low solubility, its use has been restricted to an oral form. PMM, the monodemethylated derivative, has increased solubility and antitumor activity comparable to HMM in some tumor models (5,10,28).

The clinical performance of PMM in phase I trials was disappointing because of the severity and frequency of its major toxic effects, ie, gastrointestinal and central nervous system (24,25,27,29-33). Nausea and vomiting was dose-limiting in all trials. While clearly dose-related, it occurred at all levels and became uncontrollable at higher levels. In addition, neurological toxic effects of the more commonly used antiemetics made their use very difficult once central nervous system depression became evident as a toxic manifestation of PMM. Only one partial and nine minimal responses occurred in 219 pretreated patients (24,25,27,29-33). Although PMM demonstrated activity in the Egyptian trial, it did not appear to offer a therapeutic advantage over oral HMM (34). No new trials that use PMM will be sponsored by the National Cancer Institute for the following reasons: (*a*) severity and frequency of the toxic effects, (*b*) ineffective control of the toxic effects, (*c*) overlapping toxic manifestations of commonly used antiemetics with PMM in depressing cortical function, and (*d*) no demonstrable therapeutic advantage over oral HMM. However, the interest in a clinically useful parenteral alternative to HMM continues.

At the Royal Marsden Hospital, another soluble analog of HMM, N^2,N^4,N^6-trimethyl-N^2,N^4,N^6-trimethylolmelamine (trimelamol), is in phase I clinical trials. PMM requires metabolic activation, which occurs rapidly in the mouse, but scarcely at all in man; hence, these investigators decided to exploit an activated melamine, ie, trimelamol (35). In addition, investigators at the Mayo Clinic have developed an iv formulation of HMM that includes solubilization of the drug with either ethanol or dimethylacetamide and administration in intralipid 20% (36). A phase I trial of this formulation is currently under investigation at the Mayo Clinic (37). Unless the toxic effects on either of these two ongoing trials are excessive, the two agents will be brought to phase II trials to assess clinical activity. It is hoped that one of these drugs, or alternative analogs under development, will ultimately provide a clinically useful parenteral triazine.

REFERENCES

1. BLUM RH, LIVINGSTON RB, and CARTER SK. Hexamethylmelamine—a new drug with activity in solid tumors. Eur J Cancer 9:195-202, 1973.
2. LEGHA SS, SLAVIK M, and CARTER SK. Hexamethylmelamine: an evaluation of its role in the therapy of cancer. Cancer 38:27-35, 1976.
3. D'INCALCI M, BOLIS G, MANGIONE C, ET AL. Variable oral absorption of hexamethylmelamine in man. Cancer Treat Rep 62:2117-2119, 1978.
4. KREILGARD B, HIGUCHI T, and REPTA AJ. Complexation in formulation of parenteral solutions: solubilization of the cytotoxic agent hexamethylmelamine by complexation with gentisic acid species. J Pharmacol Sci 64:1850-1855, 1975.
5. CONNORS TA, CUMBER AJ, ROSS WCJ, ET AL. Regression of human lung tumor xenographs induced by water-soluble analogs of hexamethylmelamine. Cancer Treat Rep 61:927-928, 1977.
6. WORZALLA WF, JOHNSON BM, RAMIREZ G, ET AL. *N*-Demethylation of the antineoplastic agent hexamethylmelamine by rats and man. Cancer Res 33:2810-2825, 1973.
7. WORZALLA WF, KAIMAN BD, JOHNSON BM, ET AL. Metabolism of hexamethylmelamine-ring-^{14}C in rats and man. Cancer Res 34: 2669-2674, 1974.
8. RUTTY CJ, and CONNORS TA. In vitro studies with hexamethylmelamine. Biochem Pharmacol 26:2385-2391, 1977.
9. BRINDLEY C, GESCHER A, LANGDON SP, ET AL. Studies of the mode of action of antitumor triazenes and triazines, III. Metabolism studies of hexamethylmelamine. Biochem Pharmacol 31:625-631, 1982.
10. LAKE LM, GRUNDEN EE, and JOHNSON BM. Toxicity and antitumor activity of hexamethylmelamine and its *N*-demethylated metabolites in mice with transplantable tumors. Cancer Res 35:2858-2863, 1975.
11. RUTTY CJ, and ABEL G. In vitro cytotoxicity of the methylmelamines. Chem Biol Interact 8:235-246, 1980.
12. ROSS WE, MCMILLAN DR, and ROSS CF. Comparison of DNA damage by methylmelamines and formaldehyde. JNCI 67:217-221, 1981.
13. D'INCALCI M, ERBA E, BALCONI G, ET AL. Time dependence of in vitro cytotoxicity of hexamethylmelamine and its metabolites. Br J Cancer 41:630-635, 1980.
14. BUCKLEY SM, STOCK CC, CROSSLEY ML, ET AL. Inhibition of the crocker mouse Sarcoma 180 by certain ethylenimine derivatives and related compounds. Cancer 5:144-152, 1952.
15. RUTTY CJ, CONNORS TA, NGUYEN-HOANG N, ET AL. In vitro studies with hexamethylmelamine. Eur J Cancer 14:713-720, 1978.
16. WAMPLER GL, MELETTE SJ, KUMPERMINC M, ET AL. Hexamethylmelamine (NSC-13875) in the treatment of advanced cancer. Cancer Chemother Rep 56:505-514, 1972.
17. BAKER BR, and ASHTON WT. Irreversible enzyme inhibitors. 196 active-site-directed irreversible inhibitors of dihydrofolate reductase derived from 1-(4-benzyloxy-3-chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-demethyl-*s*-triazeno and bearing a terminal phenyl sulfonate group. J Med Chem 15:945-947, 1972.
18. BROGGINI M, COLOMBO T, D'INCALCI M, ET AL. Pharmacokinetics of hexamethylmelamine and pentamethylmelamine in mice. Cancer Treat Rep 65:669-672, 1981.
19. AMES MM, POWIS G, KOVACH JS, ET AL. Disposition and metabolism of pentamethylmelamine and hexamethylmelamine in rabbits and humans. Cancer Res 39:5016-5021, 1979.
20. MORIMOTO M, GREEN D, RAHMAN A, ET AL. Comparative pharmacology of pentamethylmelamine and hexamethylmelamine in mice. Cancer Res 40:2762-2767, 1980.
21. COLOMBO T, TORTI L, and D'INCALCI M. Dose-dependent pharmacokinetics of PMM in the rat. Cancer Chemother Pharmacol 5:201-203, 1981.
22. RUTTY CJ, NEWELL DR, MUINDI JRK, ET AL. The comparative pharmacokinetics of pentamethylmelamine in man, rat, and mouse. Cancer Chemother Pharmacol 8:105-111, 1982.
23. DUTCHER JS, JONES RB, and BOYD MR. A sensitive and specific assay for pentamethylmelamine in plasma: applicability to clinical studies. Cancer Treat Rep 64:99-104,1980.
24. IHDE DC, DUTCHER JS, YOUNG RC, ET AL. Phase I trial of pentamethylmelamine: a clinical and pharmacologic study. Cancer Treat Rep 65:755-762, 1981.
25. CASPER ES, GRALLA RJ, LYNCH GR, ET AL. Phase I and pharmacological studies of pentamethylmelamine administered by 24 hour intravenous infusion. Cancer Res 41:1402-1406, 1981.
26. BENVENUTO JA, STEWART DJ, BENJAMIN RS, ET AL. Pharmacology of pentamethylmelamine in humans. Cancer Res 41:566-569, 1981.
27. MUINDI JRF, NEWELL DR, SMITH IE, ET AL. Pentamethylmelamine (PMM): phase I clinical and pharmacokinetic studies. Br J Cancer 47:027-033, 1983.
28. Clinical Brochure. Pentamethylmelamine (NSC-118742). Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, 1978.
29. EAGAN RT, CREAGAN ET, RUBIN J, ET AL. Early clinical trial of a 1-day intermittent schedule for pentamethylmelamine. Cancer Treat Rep 64:993-995, 1980.
30. VAN ECHO DA, CHIUTEN DF, WHITACRE M, ET AL. Phase I trial of pentamethylmelamine in patients with previously treated malignancies. Cancer Treat Rep 64:1335-1339, 1980.
31. AJANI JA, CABANILLAS FF, and BODEY GP. Phase I trial of pentamethylmelamine. Cancer Treat Rep 66:1227-1228, 1982.
32. GOLDBERG RS, GRIFFIN JP, MCSHERRY JW, ET AL. Phase I study of pentamethylmelamine. Cancer Treat Rep 64:1319-1322, 1980.
33. Phase I Minutes. Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, 1979.
34. GAD-EL-MAWLA N, ZIEGLER JL, HAMZA R, ET AL. Randomized phase II trial of hexamethylmelamine versus pentamethylmelamine in carcinoma of the bilharzial bladder. Cancer Treat Rep 68:793-794, 1984.
35. JUDSON IR, RUTTY CJ, ABEL D, ET AL. Phase I trial of N_2, N_4, N_6-trihydroxymethyl-N_2, N_4, N_6-trimethylmelamine (trimelamol) an antitumor *s*-triazine. Proc ASCO 1985. In press.
36. AMES MM, and KOVACH JS. Parenteral formulation of hexamethylmelamine potentially suitable for use in man. Cancer Treat Rep 66:1579-1581, 1982.
37. AMES MM. Personal communication.

Metalloporphyrin Contrast Agents for Magnetic Resonance Imaging of Human Tumors in Mice[1,2]

N. J. Patronas,[3] J. S. Cohen,[4,*] R. H. Knop,[3] A. J. Dwyer,[3] D. Colcher,[4] J. Lundy,[4] F. Mornex,[4] P. Hambright,[5] M. Sohn,[4] and C. E. Myers[4]

Enhanced contrast of transplanted human colon carcinoma in athymic mice is seen in magnetic resonance images upon iv injection of the complexes of Mn(III) with two water-soluble porphyrins, tetra (4-sulfonatophenyl) porphyrin and tetra (*N*-methyl-4-pyridyl) porphyrin. [Cancer Treat Rep 70:391–395, 1986]

A number of porphyrin derivatives are known to exhibit selective uptake by human and animal tumors (1,2). These agents also undergo photoactivation, and this forms the basis for phototherapy of several human tumors (2). While the corresponding metalloporphyrins are not photoactive, previous work using radioactive metal complexes of water-soluble porphyrins has also demonstrated varying degrees of selective retention by tumors in animals (2–5). These observations led us to test the effectiveness of water-soluble paramagnetic metalloporphyrins as contrast agents for tumors in magnetic resonance (MR) imaging (6,7).

Several paramagnetic complexes of tetra (4-sulfonatophenyl) porphyrin (TPPS) were initially chosen for testing because its ^{109}Pd derivative was found to be the most effectively retained of several water-soluble metalloporphyrins by tumors in mice (5). We have reported the effect of several paramagnetic metal ion complexes of TPPS on the relaxation times (T_1 and T_2) of water protons, and based on these results have predicted their potential usefulness as MR contrast agents (8).

The metal-TPPS complexes were found to be more efficient relaxation agents than the free metal ions. Although free paramagnetic metal ions have been used to provide contrast in MR imaging (9,10), they have no established selectivity for tumors, and are generally regarded as toxic for humans at the levels required for effective contrast (11,12). Paramagnetic metal ion chelates have been used to provide contrast in MR images, notably GdDTPA (diethylenetetramine pentaacetic acid) (13), although such chelates exhibit less efficient relaxation than the free metal ions (14). Of the metal-TPPS complexes studied, it was also found that Mn(III)-TPPS is a more efficient relaxation agent for water than the corresponding Fe(III) and Cu(II) TPPS complexes (8). We now report the use of Mn(III) porphyrin complexes as selective MR imaging contrast agents for tumors.

MATERIALS AND METHODS

Metalloporphyrins were obtained from Midcentury Chemicals, Chicago, IL, and Porphyrin Products, Logan, UT. Details of analytic results for some materials were quoted previously (8). Molecular weights were based on %N. Relaxation times (T_1 and T_2) of bulk water were measured at 20 MHz with an IBM PC20 pulse spectrometer. All measurements were performed at a temperature of 37°C, determined with a glass-encased thermocouple immersed in the sample. The sample was contained in a 10-mm tube placed inside a 12-mm tube and a total volume of 2–2.2 ml was used. Relaxation times were calculated automatically, and were reproducible to within 1% in duplicate runs. Selected values were recalculated independently and were found to be consistent.

Solutions for MR imaging were prepared by dissolving Mn-TPPS or Mn-tetra (*N*-methyl-4-pyridyl) porphyrin (TMPyP) in sterile water at a concentration of 5–30 mg/ml with vigorous shaking and/or magnetic stirring for several hours at room temperature. The pH was adjusted to approximately pH 7 with the addition of ali-

[1]Received Mar 6, 1985; revised May 17, 1985; accepted May 29, 1985.

[2]Preliminary accounts of this work were presented at the Third Conference on Magnetic Resonance in Medicine, New York, NY, Aug 13–17, 1984, pp 423–424, and at the Radiological Society of North America, Washington, DC, Nov 24–30, 1984, p 171.

[3]Diagnostic Radiology Department, Clinical Center, National Institutes of Health, Bethesda, MD.

[4]Clinical Pharmacology Branch, Clinical Oncology Program, Division of Cancer Treatment (*J. S. Cohen, M. Sohn,* and *C. E. Myers*), and Laboratory of Tumor Immunology and Biology, Division of Cancer Biology and Diagnosis (*D. Colcher, J. Lundy,* and *F. Mornex*), National Cancer Institute, Bethesda, MD.

[5]Chemistry Department, Howard University, Washington, DC.

[*] *Reprint requests to:* Dr. J. S. Cohen, Clinical Pharmacology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 6N105, National Institutes of Health, Bethesda, MD 20892.

quots of 0.1-0.5 M H_2SO_4 with magnetic stirring. The solutions were then filtered through 22-μm filters (Millipore), and filtrate concentrations were checked by visible spectroscopy using experimentally determined extinction coefficients (eg, Mn-TPPS at pH 3.0 $\varepsilon_{467} = 97.6 \times 10^3$ $mol^{-1} cm^{-1}$).

The athymic mice (nu^+/nu^+; Frederick Cancer Research Facility, MD) had been implanted sc with human colon carcinoma (LS-147T, 10^6) cells (15), which had produced malignancies of about 2 cm at approximately 6 weeks postimplantation. Ten of these animals were injected with 0.3-0.5 mg of Mn-TPPS per gram of body weight via the lateral vein of the mouse. Two additional animals were given 0.3 mg of Mn-TMPyP per gram of body weight using the same route. Mice were sedated prior to imaging with chloral hydrate (0.75%, 0.012 ml/g of body weight).

Magnetic resonance imaging was carried out on a Picker Corporation whole-body superconducting system operating at 0.26 T (10.08 MHz) using a 30-cm transmitter coil. Maximum contrast of the colon carcinoma implants was obtained with an inversion recovery (TI = 400-600 msecs; TR = 1500-1700 msecs) or with a spin echo (TE = 40 msecs; TR = 1700 msecs) image sequence. Images were constructed using a 256 × 256 matrix over a 30-cm field of view producing pixels of approximately 1.2 mm.

RESULTS

For comparison with the previous results on TPPS complexes (8), the relaxation rates of the Mn(III) and Fe(III) complexes of TMPyP and tetra (*N*,*N*,*N*-trimethyl-4-aniliniumyl) porphyrin (TAP) as a function of concentration are shown in figure 1. The Mn(III) complexes of TMPyP and TAP gave comparable relaxivity (slope) to the value previously reported for Mn-TPPS (8).The homologous Fe(III) complexes gave relaxivity of approximately half these values. We have also compared the value for the spin labeled compound TEMPO, which is much lower (fig 1).

The bulk water relaxation times of samples of excised tissues and whole tumors were also measured in mice with and without iv injection with the metalloporphyrins at a concentration of 5-10 mM in sterile water. The bulk T_1 values of the tumor were significantly decreased after injection with Mn-TPPS and Mn-TMPyP (for example, with Mn-TMPyP: untreated, 1.06 sec; treated, 0.48 sec).

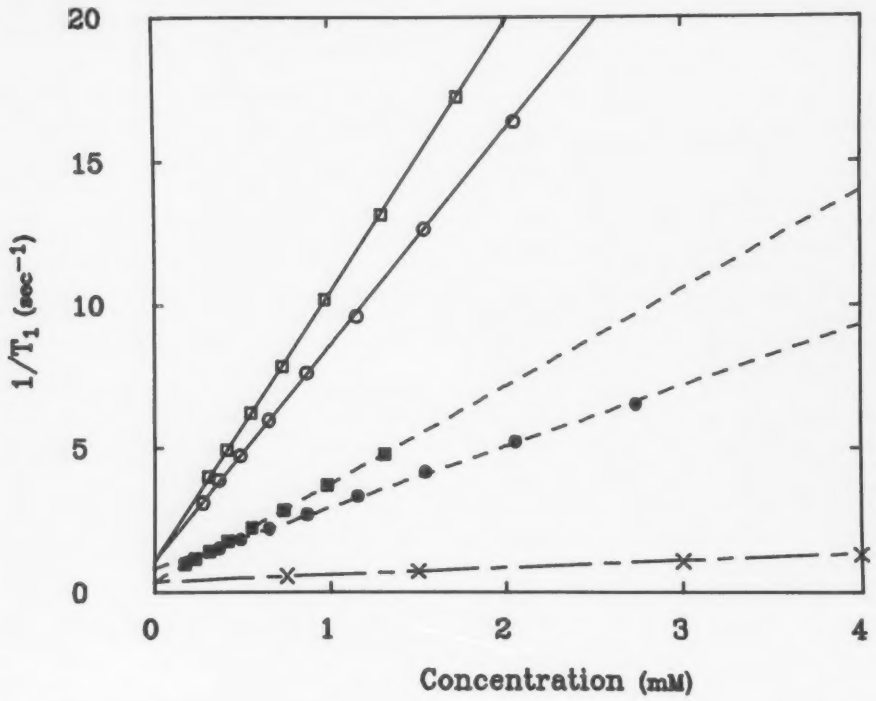

FIGURE 1.—Spin lattice relaxation rates ($1/T_1$) of water as a function of concentration (mM). □ = Mn(III)-TAP [relaxivity = 9.35 ± 0.03 $(mM\ sec)^{-1}$]; ○ = Mn(III)-TMPyP (7.50 ± 0.03); ■ = Fe(III)-TAP (3.40 ± 0.01); ● = Fe(III)-TMPyP (2.12 ± 0.03); and X = nitroxide spin label (TEMPO) (0.23 ± 0.002).

MR images of mice were taken 1 hr, 24 hrs, and in several cases, periodically up to 1 week after injection with Mn-TPPS; a total of 12 animals were injected and imaged. In all cases, remarkably enhanced contrast was observed for the colon carcinoma, and at the higher dosage levels other organs also showed shorter relaxation times, particularly the kidney, as indicated by comparative relaxation time measurements of excised tissues (16). Representative magnetic resonance images are shown in figure 2, with an untreated control present. In two experiments, a positive enhancement was similarly seen upon injection of Mn-TMPyP.

DISCUSSION

Four main factors determine the relative effectiveness of a paramagnetic contrast agent in MR imaging: (*a*) relaxation efficiency, or relaxivity on a comparative molar basis, (*b*) selective tissue uptake and/or retention, (*c*) comparative chemical and biochemical stability, and (*d*) toxicity. The relative importance of these factors will differ for different choices of contrast agent. The relative slope of plots of relaxation rate ($1/T_1$) of water versus concentration [the relaxivity in $(mM\ sec)^{-1}$] of different paramagnetic solutes provides a readily apparent means to compare their potential effectiveness as contrast agents in MR imaging (8). This is because the relaxation rate is directly proportional to the concentration of a paramagnetic species (17). However, the degree of selective uptake and/or retention by a tumor, or another specific organ, could be more important than an enhanced relaxation rate in a given case. Since intrinsic contrast between normal soft tissues in MR imaging depends on differences in relaxation rates of only factors of 2 or 3 (7), considerations of both relaxation enhancement and selective retention will become crucial in determining actual contrast achievable in MR imaging.

It has been suggested that spin labeled compounds, usually nitroxide radicals, could be useful contrast agents (18,19). However, as seen in figure 1, such compounds are much less effective than the metalloporphyrins as relaxation agents. We have measured the relaxivity of several other water-soluble nitroxide spin labeled derivatives (20), all of which gave identical values within experi-

FIGURE 2.—Inversion recovery MR images (TI = 600 msecs, TR = 1500 msecs) taken on a Picker Corp imager operating at 0.26 T (10.08 MHz) with a 30-cm coil. The 3 mice were sedated; the middle one was uninjected, the one on the right was injected with 4.1 mg of Mn(III)-TPPS, and the one on the left was injected with 8.6 mg. These images were taken 24 hrs after injection. The uppermost area of bright contrast (short relaxation time) is the human colon carcinoma xenograft.

mental error. Not only do spin labels have one unpaired electron per molecule, as opposed to four for Mn(III) complexes, but the water protons being relaxed do not directly form ligands to the spin moiety as they do in metal ion complexes. Since the relaxation effect is proportional to the inverse sixth power of the distance between the ligand protons and the electron spins, this leads to a much greater effectiveness of metal ion complexes. It should be emphasized that strongly chelating ligands which fill most ion ligation sites are expected to reduce the effectiveness of a given metal ion for relaxation of solvent water (12). Such factors as the rate of exchange (usually the off rate) for water ligands on metal ion sites are crucial for determining the relaxation efficiency of, for example, Mn(III) complexes, since rapid exchange shares and magnifies the relaxation effect throughout the aqueous solvent (12,21). These factors result in a difference of an order of magnitude in the paramagnetic relaxation effect of Mn-porphyrin complexes compared to spin labels (fig 1). An alternative strategy is to attempt to increase the selectivity of the reagent by covalently attaching spin labels to monoclonal antibodies against specific cancer cell surface markers (22). However, while such a strategy increases selectivity, it greatly reduces the total dosage of paramagnetic reagent that can be injected.

Many metalloporphyrins are well-characterized (23), including Mn(III)-porphyrin complexes (24). The Mn(III) complex with TPPS is so stable to acid that its stability constant cannot be measured (25). It has been reported that the stability of metalloporphyrins in vivo follows the order of their metal-complex stability in solution (26). Consequently, we presume that the contrast we observe in images in vivo does not arise from de-metallation of the Mn(III)-porphyrin complexes. Another indication that this is the case is the difference in tissue distribution observed in mice that have been injected with Mn-TPPS compared to $MnCl_2$ (16,27). Further studies are under way to attempt to determine the extent of residual complexation in the intact tissue.

The above results and considerations serve to focus attention on Mn(III)-porphyrins as effective contrast agents in MR imaging of human tumors in mice. It should be noted that the overall charge on the Mn-TPPS complex is negative, while those on Mn-TMPyP and Mn-TAP are positive. These preliminary results show that both reagents give enhanced contrast. Work is in progress to compare relative degrees of enhancement realizable with these and other (28) paramagnetic metalloporphyrins. It has been reported that the free porphyrin TPPS is a neurotoxin (29). Subsequent work must address the related questions of toxicity and in vivo stability if paramagnetic metalloporphyrins are to be successfully used as MR contrast agents in man.

REFERENCES

1. DOIRON DR, and GOMER CJ, eds. Porphyrin Localization and Treatment of Tumors. New York, A. Liss, 1984.
2. KESSEL D. Porphyrin localization: a new modality for detection and therapy of tumors. Biochem Pharmacol 33:1389-1393, 1984.
3. WINKELMANN J, SLATER G, and GROSSMAN J. The concentration in tumor and other tissues of parenterally administered tritium and ^{14}C labelled tetraphenylporphine sulfonate. Cancer Res 27:2060-2064, 1967.
4. ZANELLI GD, and KAELIN AC. Synthetic porphyrins as tumor localizing agents. Br J Radiol 54:403-407, 1981.
5. HAMBRIGHT P, FAWWAZ R, VOLK P, ET AL. The distribution of various water soluble radioactive metalloporphyrins in tumor bearing mice. Bioinorg Chem 5:87-92, 1975.
6. MARGULIS AR, HIGGINS CB, KAUFMAN L, ET AL. Clinical Magnetic Resonance. San Francisco, University of California, 1983.
7. BUDINGER TE, and LAUTERBUR PC. Nuclear magnetic resonance technology for medical applications. Science 226:288-298, 1984.
8. CHEN C, COHEN JS, MYERS CE, ET AL. Paramagnetic metalloporphyrins as potential contrast agents in NMR imaging. FEBS Lett 168:70-74, 1984.
9. MENDOCA DMH, LAUTERBUR PC, and RUDIN AM. *In* Proceedings of the First Conference on Magnetic Resonance in Medicine, Boston, 1982, pp 103-104.
10. GOLDMAN MR, BRADY TJ, PYKETT IL, ET AL. Applications of NMR imaging to the heart. Radiology 142:246-247, 1982.
11. VENUGOPAL B, and LUCKEY TD. Metal Toxicity in Mammals. New York, Plenum, 1978.
12. WOLF GL, and BAUM L. Cardiovascular toxicity and tissue proton T_1 response to manganese injection in the dog and rabbit. Am J Radiol 141:193-197, 1983.
13. Proceedings of the Third International Conference on Magnetic Resonance in Medicine. New York, Aug 13-17, 1984.
14. KANG YS, GORE JC, and ARMITAGE IM. Studies of factors affecting the design of contrast agents: manganese in blood as a model system. Mag Resonance Med 1:396-409, 1984.
15. TOM BH, RUTZKY LP, JAKSTYS MM, ET AL. Human colonic adenocarcinoma cells. I. Establishment and description of a new cell line. In Vitro 12:180-191, 1976.
16. LYON R, FAUSTINO P, MORNEX F, ET AL. Unpublished results.
17. BLOEMBERGEN N, PURCELL EM, and POUND RV. Relaxation effects in nuclear magnetic absorption. Phys Rev 73:679-712, 1948.
18. BRASCH RC. Work in progress: methods of contrast enhancement for NMR imaging and potential applications. Radiology 147:781-788, 1983.
19. BRASCH RC, NITECKI DE, BRANT-ZAWADSKI M, ET AL. Brain nuclear magnetic resonance imaging enhanced by a paramagnetic nitroxide contrast reagent. Am J Radiol 161:1019-1023, 1983.
20. PLESSAS NR, and GOLDSTEIN IJ. Synthesis of α and βC glycosides as probes for studies of carbohydrate-protein interactions. Carbohydr Res 89:211-220, 1981.
21. KOENIG SH, and BROWN RD. Relaxation of solvent protons by paramagnetic ions and its dependence on magnetic field and chemical environment: implications for NMR imaging. Mag Res Med 1:478, 1984.
22. MCNAMARA MT, WESBEY GE, EHMAN RL, ET AL. Selective alterations in tumor relaxation times using a paramagnetic labelled monoclonal antibody to Burkitt's lymphoma. *In* Proceedings of the International Conference on Magnetic Resonance in Medicine, 1984, pp 514-515.
23. SMITH KM, ed. Porphyrins and Metalloporphyrins. New York, Elsevier, 1975.
24. BOUCHER LJ. Manganese porphyrin complexes. Coord Chem Rev 7:289-329, 1972.

25. HAMBRIGHT P. An acid solvolysis kinetic study of Mn(II) tetrakis(*N*-methyl-3-pyridyl) porphyrin. J Inorg Nucl Chem 39:1102–1104, 1977.
26. WANG TST, FAWWAZ RA, and THOMASHEFSKY P. Metalloporphyrin derivatives: structure-localization properties. *In* Radiopharmaceuticals: Structure-Activity Relationships (Spencer RP, ed). New York, Grune and Stratton, 1981, pp 225–249.
27. MAYNARD LS, and COTZIAS GC. The partition of manganese among organs and intracellular organelles of the rat. J Biol Chem 214:489–495, 1955.
28. JACKSON LS, NELSON JA, CASE TA, ET AL. Manganese protoporphyrin. IX. A potential intravenous paramagnetic NMR contrast agent. Personal communication.
29. RIOPELLE RJ, and KENNEDY JC. Experimental porphyric neuropathy. Can J Neurol Sci 8:105–114, 1981.

Growth Inhibition and Eicosanoid Metabolism in the R3230AC Mammary Adenocarcinoma by Interferon[1,2]

Rashida A. Karmali,[3,*] Huub Schellekens,[4] Frank Lefevre,[5] Jane Marsh,[5] Charles Fuchs,[5] and Mathilde Krim[5]

The mode of action of rat interferon (IFN) on growth of the R3230AC mammary adenocarcinoma was studied in vivo in Fischer female rats. A dose of 1×10^4 units of rat IFN given thrice weekly inhibited the growth of the transplanted mammary tumors. Of the five eicosanoids measured in the tumor, the content of four arachidonate products, prostaglandin (PG) E_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and thromboxane (TX) B_2, was higher in mammary tumors from IFN-treated rats than the control rats. PGE_2 was the major eicosanoid. In vitro PG synthesis (PGE_1, PGE_2, and $PGF_{2\alpha}$) was lower in tumor microsomes prepared from IFN-treated tumors. These data suggest that the tumor content of four arachidonate products in the IFN-treated tumors was related to the in vivo effects of rat IFN. Indomethacin, a cyclooxygenase inhibitor, also inhibited tumor growth. Furthermore, when indomethacin was administered daily in combination with rat IFN, the tumor-inhibiting effect of rat IFN was reduced. These observations suggest that the effects of eicosanoids appear to be biphasic in this tumor model. Inhibition of arachidonic acid metabolism resulted in tumor growth inhibition, a finding consistent with the view that eicosanoid production is required for tumor enhancement. Conversely, in the experiments with rat IFN, retardation of tumor growth is associated with a greater amount of arachidonic acid metabolism, and indomethacin prevents this effect. Eicosanoids appear to be required for tumor-inhibiting effects of rat IFN, and yet inhibition in vivo of eicosanoid synthesis also resulted in retardation of tumor growth. Although the precise mechanism of action in each situation is unclear, these apparently contradicting results are consistent with the biphasic actions of eicosanoids reported in some normal tissues and transformed tumor cell lines. [Cancer Treat Rep 70:397–400, 1986]

The interferons (IFNs) have been shown to exert a wide variety of biologic actions in addition to conferring cell resistance to virus infection and inhibiting growth of malignant cells in vivo and in vitro (1–4). Such a range of functions suggests the involvement of intermediary or secondary messenger molecules.

IFN and IFN inducers can stimulate eicosanoid production (5), and a direct link between IFN induction and eicosanoid production in the virus-infected cell cultures was observed (1). Eicosanoids are known to have various effects on physiological and biochemical events, and often they affect the same processes which are influenced by IFN. Therefore, eicosanoids may represent a potential system for mediating or modulating the effect of IFN. This hypothesis is supported by studies of eicosanoid synthesis by cell lines cultured in vitro. Mouse leukemia cells resistant to IFN were found to be devoid of fatty acid cyclooxygenase activity (6), and human IFN-β stimulated the synthesis of prostaglandin (PG) E and $PGF_{2\alpha}$ in IFN-sensitive human cell lines but not in IFN-resistant cells (7). Although several reports have shown that IFN stimulates the synthesis of eicosanoids by cell lines in vitro, there is as yet no information on the effect of IFN on tumor eicosanoid production in vivo and the evaluation of these changes in relation to tumor growth.

In this study, we investigated the effect of rat IFN on growth of the R3230AC mammary adenocarcinoma in rats; eicosanoid content (ng/g of wet tissue) and eicosanoid synthesis by tumor microsomes (ng/mg of protein/10 mins) were measured. The effect of indomethacin (an in-

[1]Received Oct 26, 1983; revised June 13, 1985; accepted July 15, 1985.

[2]Supported by grant PDT-206 from the American Cancer Society.

[3]Department of Nutrition, Cook College, Rutgers, The State University of New Jersey, New Brunswick.

[4]REP-Institutes of the Organization for Health Research, Rijswijk, The Netherlands.

[5]Memorial Sloan-Kettering Cancer Center, New York, NY.

[*]*Reprint requests to:* Dr. R. A. Karmali, Department of Nutrition, Rutgers, The State University of New Jersey, PO Box 231, New Brunswick, NJ 08903.

hibitor of cyclooxygenase) on IFN-induced changes in tumor growth was also studied.

MATERIALS AND METHODS

Tumor.—The R3230AC mammary adenocarcinoma, an estrogen-responsive tumor (8), was obtained from Mason Research Institute, Worcester, MA. Tumor material for transplantation was prepared from a freshly dissected tumor which was placed in medium 199 (Grand Island Biological Company, NY) and cut into approximately uniform pieces of about 3 mm^3. One piece was transplanted sc in each animal.

Animals.—Female Fischer 344 rats (Charles River Breeding Laboratory, Kingston, NJ) weighing 70–100 g at the start of the experiment were housed in groups of 15 under standard laboratory conditions and fed rat chow and water ad libitum.

Treatment.—In the first protocol, 0.25 ml containing 10^4 units of rat IFN with relative specific activity of about 10^6 units/mg of protein (9) was injected sc in 15 rats three times a week. Fifteen control rats were injected with 0.9% saline. At the end of 3 weeks the tumors were dissected, weighed, measured, and frozen rapidly at -70°C for PG studies. In the second protocol, four groups were used: control (0.9% saline); rat IFN (10^4 units); rat IFN (10^4 units) + indomethacin (5 mg/kg of body weight); and indomethacin (5 mg/kg of body weight). Indomethacin, an inhibitor of cyclooxygenase, was given orally. The treatments were started 2 days before the tumors were transplanted and continued for 3 weeks. At the end of the experiment, the animals were killed and the tumors were dissected out, measured, and weighed.

Eicosanoid Measurement Procedures

Materials.—PG standards PGE_1, PGE_2, $PGF_{2\alpha}$ tromethamine salt, 6-keto-$PGF_{1\alpha}$, and TXB_2 were kindly supplied by Dr. J. Pike (Upjohn Co, Kalamazoo, MI). Tritium-labeled compounds were purchased from New England Nuclear (Boston, MA). Rabbit antisera to PGE_1, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TXB_2 were raised in our laboratory. The cross-reactivities of these antibodies for the nontargeted eicosanoids were not > 4%, except for PGE_1 and PGE_2 antisera which cross-reacted 10% with PGE_2 and PGE_1 standards, respectively. Unlabeled sodium arachidonate (grade 1) was obtained from Sigma (St. Louis, MO).

Analytical methods: extraction.—The procedure for extracting the eicosanoids was described earlier (10). Briefly, a trace of ^{3}H-PG was added to aliquots of standards and samples before being extracted once with 3.5 ml of petroleum ether. After acidification to pH 3.5, the samples were extracted twice with diethyl ether, dried under nitrogen, and reconstituted in assay buffer. The efficiency of the extraction procedure to this point was 85%–95%.

Radioimmunoassay.—Standard quantities of each PG (0–1000 pg) or the unknown extracted sample to be measured were prepared in 0.1-ml aliquots of assay buffer. Antiserum and label were added successively in 0.1-ml aliquots and incubated at 4°C for 8–12 hours. Bound and free ^{3}H-PG were separated by 0.5 ml of dextran-coated charcoal (0.5%–1.0% by weight) to estimate the amount of each compound in the unknown samples. The sensitivity of the assays has been found to be approximately 10 pg. The intra-assay coefficient of variation was 9.0%.

Assay of eicosanoids in mammary tissue.—Solid tumor fragments weighing between 0.5 and 1 g were ground using a mortar and pestle in a 1:5 (g:ml) tissue:buffer volume of MES buffer (1 M 2n-morpholino-ethane sulfonic acid, pH 7.4, containing 2 mM $CaCl_2$, 2% glycerol, and 1 mM monothio-glycerol) (11). Addition of an antioxidant was omitted to enable the isolation of unaltered PG synthetase for in vitro activity tests. Homogenates were centrifuged at 800 × *g* for 15 minutes at 4°C. The supernatant thus obtained was further spun at 150,000 × *g* for 1 hour at 4°C. The subsequent supernatant was collected and stored in duplicate at -20°C. ^{3}H-PGE_2 was added as a tracer and PGs were extracted from this sample and measured by radioimmunoassay (RIA) as described above. Such measurements represented the yield of the five immunoreactive compounds in tumor tissues. The remaining microsomal pellet obtained after centrifugation at 150,000 × *g* was saved to carry out studies of PG synthetase activity in vitro.

Eicosanoid synthetase assay.—The microsomal pellet was suspended in MES buffer and its protein content was measured by the method of Lowry et al (12). The final concentration of the microsomal fraction was adjusted to 0.5 mg of protein/ml in MES buffer. Biosynthesis of eicosanoids by microsomal preparations of neoplastic mammary tissue was assayed by a procedure described earlier (11).

A 0.2-ml aliquot of the microsomal fraction was incubated at 37°C with 0.8 ml of MES buffer containing 1.25 mM epinephrine, 1.25 mM reduced glutathione, and 1.25 μm of sodium arachidonate. After an incubation period of 10 minutes, tritiated PGE_2 (1200 cpm) was added as a tracer to the above mixture to evaluate procedural losses. Eicosanoids were extracted with a petroleum ether/diethyl ether mixture as described in the *Analytical methods: extraction* section. The organic extracts were then dried under nitrogen and taken up in buffer for subsequent measurement by RIA of PGE_1, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TXB_2.

Statistical analysis.—Student's t test was used to compare each group against the control, and analysis of variance was applied to test the relationship with effect of IFN treatment.

RESULTS

Inhibition of Tumor Growth With Rat IFN

Administration (sc) of rat IFN (1×10^4 units/rat) three times a week caused a small (38%) but significant reduction in tumor weight; tumor volume was also inhibited significantly (41%) (table 1). There was no difference in body weight between control and IFN-treated groups.

Increase of Tumor Arachidonate Products With IFN Therapy (ng/g of Wet Tissue)

The levels of five eicosanoids in the tumor tissue were measured: PGE_1, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TXB_2. The content of the four arachidonate metabolites was higher in mammary tumors from IFN-treated rats compared to those from control rats (table 2). PGE_2 was the major arachidonate metabolite in both the saline- and IFN-treated groups. There was no significant difference in tumor PGE_1 content between the two groups.

In Vitro Eicosanoid Synthesis (ng/mg Protein/10 Mins)

When tumor microsomes were incubated in vitro with appropriate cofactors, PGE_1, PGE_2, and $PGF_{2\alpha}$ synthesis was lower in the IFN-treated mammary tumors than in the saline-treated group (table 3). PGE_2 was the major prostanoid. There was no significant difference in 6-keto-$PGF_{1\alpha}$ and TXB_2 synthesis.

Effect of Indomethacin Treatment on Tumor Growth

IFN treatment inhibited both the tumor weight and volume as before (table 4). However, when indomethacin (5 mg/kg of body weight) was administered daily in addition to the IFN treatment (thrice weekly), there was no significant effect on tumor growth. Tumor growth in the group treated with indomethacin alone was inhibited.

DISCUSSION

The inhibitory effects of IFN on tumor growth have been shown in a number of experimental tumor models (13,14). The results in the present study show that treatment of tumor-bearing rats thrice weekly with 1×10^4 units of rat IFN significantly inhibited the growth of the R3230AC mammary adenocarcinoma.

The tissue levels of all four arachidonate products examined were significantly elevated in the IFN-treated tumors compared with control mammary tumors. PGE_2 was the major eicosanoid both in the saline- and IFN-treated groups. While previous studies have shown that IFN stimulates eicosanoid synthesis by tumor cell lines in vitro, this study additionally demonstrates elevated levels of four arachidonate products as a result of IFN treatment in vivo.

On the other hand, tumor PG synthetase activity in vitro was lower in the IFN-treated group compared with the control tumor microsomes. This difference between in vivo and in vitro PG synthesis may be due to a number of reasons: since exogenous IFN was not added to in vitro cultures of tumor microsomes, it would be expected that eicosanoid synthesis would be comparable in both the groups. Instead, synthesis of PGE_1, PGE_2, and $PGF_{2\alpha}$ was significantly smaller by tumor microsomes from IFN-treated rats than control rats. These results suggest that IFN treatment administered in vivo to tumor-bearing rats resulted in a reduced tumor PG synthetase activity. Since tumor content of four arachidonate products was actually greater in the IFN-treated rats, these results indicate that a significant portion of tumor eicosanoids was either made by cells other than the tumor cell, eg, infiltrating polymorphonuclear cells, and/or a result of a slower removal or breakdown of parent compounds in the IFN-treated group.

TABLE 1.—Effect of rat IFN on body weight, tumor weight, and tumor volume*

	Body weight (g)	Tumor weight (g)	Tumor volume (cm^3)
Control (0.9% saline)	134	5.2 ± 0.5	5.4 ± 0.47
Rat IFN	136	3.2 ± 0.5 ($P = 0.015$)	3.2 ± 0.49 ($P = 0.003$)

*Values for tumor weight and tumor volume = mean ± SE. Fischer 344 female rats were inoculated sc with 1×10^4 units of rat IFN thrice weekly. Statistical analysis: Student's t test ($n = 15$).

TABLE 2.—Tumor PG content (ng/g of wet tissue)*

	Control	Rat-IFN
PGE_1	5.6 ± 2.2	4.6 ± 0.8
PGE_2	88.0 ± 28.3	131.8 ± 29.3
$PGF_{2\alpha}$	1.4 ± 1.0	3.3 ± 0.7 ($P = 0.023$)
6-Keto-$PGF_{1\alpha}$	3.1 ± 1.0	40.6 ± 7.1 ($P < 0.001$)
TXB_2	1.7 ± 0.4	11.7 ± 2.4 ($P = 0.001$)

*Values = mean ± SE. Tumors were processed in MES buffer at 2°C as described in the Materials and Methods section; 0.2 ml of the supernatant was used to measure prostaglandins. Statistical analysis: Student's t test ($n = 15$).

TABLE 3.—PG synthesis by tumor microsomes in vitro (ng/mg of protein/10 mins)*

	Control	Rat-IFN
PGE_1	30.7 ± 1.6	7.1 ± 0.7 ($P < 0.001$)
PGE_2	137.5 ± 8.0	94.9 ± 12.0 ($P = 0.007$)
$PGF_{2\alpha}$	5.0 ± 0.6	3.3 = 0.6 ($P = 0.043$)
6-Keto-$PGF_{1\alpha}$	3.2 ± 0.6	4.8 ± 0.7
TXB_2	2.8 ± 0.3	3.0 ± 0.3

*Values = mean ± SE. 0.1 mg of microsomal protein was incubated with appropriate cofactors for 10 mins at 37°C. PG measurements were carried out as described in the Materials and Methods section. Statistical analysis: Student's t test ($n = 15$).

TABLE 4.—Effect of IFN and indomethacin on growth of the R3230AC mammary adenocarcinoma *

	Tumor weight (g)	Tumor volume (cm^3)
Control	2.5 ± 0.30	2.3 ± 0.30
IFN (1×10^4 units)	1.7 ± 0.40	2.0 ± 0.64
IFN + indomethacin (5 mg/kg of body weight)	2.2 ± 0.27	1.9 ± 0.22
Indomethacin (5 mg/kg of body weight)	2.0 ± 0.11	1.7 ± 0.11 ($P = 0.023$)

* Values = mean ± SE. Rat IFN (1×10^4 units) was inoculated sc 3 times/wk; indomethacin (5 mg/kg of body weight) was administered orally each day. Control and indomethacin groups comprised 15 rats each; IFN and IFN + indomethacin groups comprised 5 rats each due to a shortage of IFN supplies. Statistical analysis: Student's t test (treated vs control).

It has been suggested that eicosanoids may represent a potential system for mediating or modulating the effect of IFN (5-7). We tested this hypothesis by studying the effect of IFN therapy on mammary tumor growth in combination with indomethacin, an inhibitor of cyclooxygenase. Tumor-inhibiting effect of IFN (1×10^4 units) was reduced when IFN was given in combination with indomethacin. These results suggest that eicosanoids may play an important role in the mediation of IFN action. However, interpretation of their role and function is complicated by the observation that tumor growth was also inhibited in the group treated with indomethacin alone, thus suggesting that inhibition of eicosanoid synthesis may also result in tumor inhibition. In this regard, it is important to define the physiologic actions and effects of eicosanoids in relation to the biology of neoplasia.

Excessive PG production, particularly of the E series, has been reported in several types of malignant tissues, including mammary tumors in both humans and experimental animals. Published reports suggest both a dose-dependent stimulatory and an inhibitory role for PGs in cultures of tumor cell lines and in experimental tumor systems (15-17). The suggestion is that low PG levels are required for cell proliferation and tumor growth, while high levels will inhibit tumor growth. If this view is correct, increasing the PG production by stimulation with IFN may inhibit the growth of mammary tumors. It is important to note that several intervening variables complicate interpretation of eicosanoid studies in tumor systems (11). The present study is concerned primarily with the possible role of eicosanoids in the mediation of IFN actions.

When IFN has been injected in virus infection or cancer, patients have commonly reacted with headache, general malaise, and fever (18). Some workers thought these effects were due to impurities, but similar symptoms were seen after injection of highly purified IFNs. High serum levels of eicosanoids have been measured during natural and pyrogen-induced fever, and some medications taken to lower the body temperature (eg, aspirin) are inhibitors of eicosanoid synthesis (19). In patients receiving IFN therapy, these symptoms subside rapidly and can be prevented with aspirin, an inhibitor of cyclooxygenase. The results in the present study raise some questions. Further studies will be necessary to elucidate the role played by eicosanoids in the mechanism of IFN action in vivo.

REFERENCES

1. FRIEDMAN RM. Antiviral activity of interferons. Bacteriol Rev 41:543-567, 1977.
2. KUWATA T, FUSE A, and MORINAGA N. Effects of interferon on cell and virus growth in transformed human cell lines. J Gen Virol 33:7-15, 1976.
3. KRIM M. Review: toward tumor therapy with interferons. Part I. Interferons: production and properties. Blood 55:711-721, 1980a.
4. KRIM M. Review: toward tumor therapy with interferons. Part II. In vivo effects. Blood 55:875-884, 1980b.
5. FITZPATRICK FA, and STRINGFELLOW DA. Virus and interferon effects on cellular prostaglandin biosynthesis. J Immunol 125:431-437, 1980.
6. CHANDRABOSE KA, CUATRECASES P, POTTATHIL R, ET AL. Interferon-resistant cell line lacks fatty acid cyclooxygenase activity. Science 212:329-331, 1981.
7. FUSE A, MAHMUD I, and KUWATA T. Mechanism of stimulation by human interferon of prostaglandin synthesis in human cell lines. Cancer Res 42:3209-3214, 1982.
8. COHEN ND, and HILF R. Influence of insulin on estrogen-induced responses in the R3230AC mammary carcinoma. Cancer Res 35:560-567, 1975.
9. SCHELLEKENS M, DE WILDE GA, and WEIMAR W. Production and initial characterization of rat interferon. J Gen Virol 46:243-247, 1980.
10. KARMALI RA, MUSE P, ALLEN G, ET AL. Macrophage production of prostaglandins. Effects of fetal calf serum and diazepam: use of an improved method for extracting 6-keto-$PGF_{1\alpha}$. Prostaglandins Leukotrienes Med 8:565-577, 1982.
11. KARMALI RA, THALER HT, and COHEN L. Prostaglandin concentrations and prostaglandin synthetase activity in *N*-nitrosomethylurea-induced rat mammary adenocarcinoma. Eur J Cancer Clin Oncol 19:817-823, 1983.
12. LOWRY OH, ROSEBROUGH NJ, FARR AL, ET AL. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275, 1951.
13. GRESSER I, and BOURALI-MAURY C. Inhibition by interferon preparations of a solid malignant tumor and pulmonary metastases in mice. Nature New Biol 236:78-79, 1972.
14. CRANE JL, JR, GLASGOW LA, KERN ER, ET AL. Inhibition of murine osteogenic sarcomas by treatment with type I or type II interferon. J Natl Cancer Inst 61:871-874, 1978.
15. BENNETT A. Prostaglandins and cancer. *In* Practical Applications of Prostaglandins and Their Synthesis Inhibitors (Karim SMM, ed). Lancaster, MTP Press, 1979, pp 149-188.
16. HILLYARD LA, and ABRAHAM S. Effect of dietary polyunsaturated fatty acids on growth of mammary adenocarcinomas in mice and rats. Cancer Res 39:4430-4437, 1979.
17. KARMALI RA. Prostaglandins and cancer. Prostaglandins Med 5:11-28, 1980.
18. SCOTT GM, SECHER DS, FLOWERS D, ET AL. Toxicity of interferon. Br Med J [Clin Res] 282:1345-1348, 1981.
19. VEALE WL, and COOPER KE. Evidence for the involvement of prostaglandins in fever. *In* Recent Studies of Hypothalamic Function (Lederis K, and Cooper KE, eds). Basel, Karger, 1974, pp 359-370.

Minimal Surgical Debridement for the Treatment of Chemotherapeutic Agent-Induced Skin Extravasations[1]

Timothy S. Loth[2,3,*]

A rat model was employed to determine the efficacy of early minimal debridement in decreasing the size of chemotherapeutic agent-induced skin ulcers. After receiving bilateral flank injections of doxorubicin in a concentration comparable to that received by humans, the right injection site underwent minimal debridement 1, 24, or 72 hours after the injection. The left side of each animal was not operated upon and served as a control. Three subgroups of the 1-, 24-, and 72-hour debridement groups were treated as previously described, except that any residual necrotic tissue was debrided 1 week after the original debridement. All minimal debridement and redebridement groups produced significant decreases in ulcer size compared to controls. Comparing minimal debridement and redebridement groups to controls, it was found that surgery produced reductions in the number of persistent ulcers in all groups. Minimal debridement followed by redebridement was significantly more effective than minimal debridement alone in decreasing ulcer size acutely. One case is presented in which an extravasation of dactinomycin was treated by early minimal debridement. The patient had an uncomplicated postoperative course, and at follow-up 21 months after surgery had excellent functional and cosmetic results. Based upon animal studies and human experience, early surgical debridement appears to be a promising new method for the treatment of clinically significant vesicant extravasations in humans. [Cancer Treat Rep 70: 401–404, 1986]

The complications and long-term morbidities of cutaneous ulcers caused by extravasations of vesicant antineoplastic agents have been well-documented (1,2). Ulcers from human vesicant extravasations develop slowly over 7–10 days and can continue to enlarge for 2–3 months. They have red, painful edges, tend not to heal spontaneously, and often extend to tendinous and neurovascular structures, leading to functional loss. These patients often present to the hand surgeon with established joint contractures and infected ulcers.

An aggressive approach to vesicant extravasation is supported by the experience of Linder et al (2). They described a remarkable series of significant morbidities in 40 patients with doxorubicin extravasation ulcers. Ten patients presented with sepsis, probably secondary to infection of open ulcer sites. There were seven nerve compression syndromes, with three permanent nerve deficits. Eleven patients had residual sympathetic dystrophies of the affected extremities, and nearly all of their patients developed permanent stiffness of joints of the upper extremity.

Estimates of the frequency of extravasations of chemotherapeutic agents range from 0.5 to > 6%, representing a clinically significant incidence (3–5). Attempts to prevent ulcers at sites of extravasation have ranged from topical and injectable antidotes to early surgical debridement of extravasation sites. In the only study to directly compare early surgical debridement to control and antidote groups in an animal model, we found surgery to be the most effective method of decreasing ulcer size acutely and of decreasing the number of persistent ulcers at extravasation sites (1).

In the aforementioned study, complete debridement of infiltrated cutaneous and subcutaneous tissues was performed with excellent results. Realizing that there may be some hesitation among most surgeons lacking experience evaluating antineoplastic agent extravasations to perform an early thorough debridement of an extravasation site, a more conservative initial debridement was felt to be desirable. The purpose of this study was to evaluate the efficacy of early minimal surgical debridement in the management of vesicant chemotherapeutic agent extravasations, in an attempt to develop a practical method of managing vesicant extravasations.

[1]Received Feb 28, 1985; revised June 24, 1985; accepted July 19, 1985.

[2]Orthopedic Service, Irwin Army Community Hospital, Fort Riley, KS.

[3]I thank Dr. Donald G. Corby for help with statistical analysis, and Lawrence Jones for technical support in the animal laboratory.

*Reprint requests to: Timothy S. Loth, MD, Orthopedic Service, Irwin Army Community Hospital, Fort Riley, KS 66442.

MATERIALS AND METHODS

A rat model for chemotherapeutic agent extravasations, similar to the standard model developed by Rudolph et al (6), was used in this study. Sixty Holtzman white rats ranging from 170 to 250 g in weight were housed in separate cages, receiving a diet of standard lab rat chow and water ad libitum. These animals were divided into six treatment groups with ten animals in each group.

Following induction of satisfactory anesthesia by peritoneal injection of pentobarbital (50 mg/kg), the flanks of each rat were shaved with hair clippers, and 25-gauge needles were used to inject 0.3 ml of doxorubicin hydrochloride intradermally into prepared areas bilaterally. Doxorubicin was diluted to a concentration equivalent to that received by humans during iv infusions as recommended by the manufacturer (1.5 mg/ml).

The animals underwent minimal debridement of the right flank extravasation site 1, 24, or 72 hours after doxorubicin injection. The left side was not operated on and served as a control. The debridement consisted of a 2-3-cm longitudinal incision over the center of the site of extravasation. Although it was evident that epidermis was grossly infiltrated with doxorubicin, the maximum amount of skin removed from each site never exceeded 1 × 4 mm. Attention was primarily focused upon removal of doxorubicin-infiltrated dermal and subcutaneous tissue. After this was accomplished, the wound was thoroughly irrigated with 100 ml of Ringer's lactate solution and then closed with surgical staples at 3-5-mm intervals. Three subgroups of the 1-, 24-, and 72-hour debrided groups underwent redebridement of any residual necrotic tissue at the previously debrided sites 1 week after the initial debridement.

Ulcer size was measured 5, 14, and 35 days after vesicant injection. The ulcer size determined 2 weeks after vesicant injection, a point at which most ulcers had stopped enlarging, was considered an accurate index of the acute effectiveness of therapeutic intervention in decreasing ulcer size. Paired or unpaired Student's t tests were used in ulcer size comparisons among all groups. The number of ulcers present 35 days after vesicant injection for each group was divided by the total number of injection sites in each group, thereby deriving a percentage of persistent ulcers. These percentages were compared among the control and surgical groups using Fisher's exact test (one-tail) to determine the effectiveness of treatment in facilitating resolution of these lesions by 5 weeks. P values < 0.05 were considered significant for all comparisons.

RESULTS

Necrotic skin ulcers developed at sites of doxorubicin injection within 3-7 days. These ulcers persisted in nearly 100% of the control sites for the full duration of the study, 35 days. In contrast, although ulcers did develop in the majority of the minimal debridement sites after surgery, they were significantly smaller than those in the control groups (table 1), and many spontaneously resolved within the duration of the study (table 2). Significant decreases in ulcer size compared to controls were noted in all minimal debridement and redebridement groups (table 1).

Redebridement of necrotic skin 1 week after initial minimal debridement produced a further significant decrease in doxorubicin-induced ulcer size, when compared to sites undergoing the single minimal debridement (table 3). Although mean ulcer size tended to be smaller the earlier the debridement was performed for the single and redebridement groups, respectively, the differences were not statistically significant.

All minimal surgical debridement groups had a significantly reduced number of persistent ulcers compared to controls, except for 24-hour minimal debridement, which had fewer ulcers than controls, but not to a statistically significant degree (table 2). Although redebridement was more effective than a single debridement in reducing the number of persistent ulcers at 35 days, only the 72-hour group was significantly better (table 2).

DISCUSSION

From our experience and that of others, it is evident that antineoplastic agent-induced ulcers produce morbidity of such severity as to justify aggressive methods of early treatment to prevent or at least limit their development (2). The early treatment of vesicant extravasations, however, is controversial and has recently been reviewed (1). Loth and Eversmann (1) compared early surgical debridement of extravasation sites to standard antidotes and controls in an animal model to attempt to identify an effective method of preventing ulcers or at least decreasing their size. Simulated vesicant extravasations of doxorubicin, vincristine, dactinomycin, mitomycin, and carmustine were treated with surgical debridement 1, 24, or 72 hours after vesicant injections, immediate applications of injectable antidotes (hydrocortisone, propranolol, isoproterenol, and sodium bicarbonate followed by

TABLE 1.—Comparison of doxorubicin ulcer size between minimal surgical debridement and control groups 2 wks following vesicant injections

Time from injection to debridement (hrs)	Control site: Mean diameter of necrosis ± SD(mm)	Debrided site: Mean diameter of necrosis ± SD(mm)
1	8.3 ± 1.6	4.7 ± 2.1 ($P < 0.0001$)
24	9.1 ± 3.8	6.1 ± 2.3 ($P < 0.005$)
72	7.6 ± 1.0	5.3 ± 2.9 ($P < 0.05$)
1 (Redebridement*)	9.4 ± 1.1	1.2 ± 1.3 ($P < 0.0001$)
24 (Redebridement*)	9.0 ± 1.5	1.8 ± 3.0 ($P < 0.0001$)
72 (Redebridement*)	7.9 ± 1.9	2.0 ± 2.1 ($P < 0.0005$)

* Any necrotic areas remaining after 1 wk were excised.

TABLE 2.—Comparison* between minimal surgical debridement and control groups evaluating % of persistent unhealed ulcers 35 days after vesicant injection

Interval from injection to debridement (hrs)	% unhealed control sites	% unhealed debrided sites
1	100	10 ($P < 0.0001$)
24	89	56 (NS)†
72	100	56 ($P < 0.05$)
1 (Redebridement)	100	0 ($P < 0.0001$)
24 (Redebridement)	100	11 ($P < 0.0001$)
72 (Redebridement)	100	0 ($P < 0.0001$)‡

*Fisher's exact test (1-tailed).

†NS = not significant, ie, $P > 0.05$.

‡Comparison of % of persistent ulcers among the single and redebridement groups showed only the 72-hr redebridement group to be significantly better.

hydrocortisone), or a topical antidote (DMSO-vitamin E). Of the interventions tested, early surgical debridement, even if delayed up to 72 hours, resulted in highly statistically significant reductions in vesicant ulcer size and significant improvement in ulcer healing compared to the control and antidote groups. As such, early surgical debridement was suggested as a therapeutic alternative for significant vesicant extravasations in humans.

In the aforementioned study, a thorough debridement was employed, consisting of excision of the extravasation site, including all affected cutaneous and subcutaneous tissue. This technique resulted in the prevention of ulcer development in a majority of sites treated. Recognizing that many surgeons lack experience in evaluating and debriding chemotherapeutic agent extravasations before necrosis has become extensive, a method was sought to produce acceptable results, regarding reduction in ulcer size and rapidity of ulcer resolution, with less aggressive initial debridement. It was felt that such an approach could be employed with less potential for inadvertent injury than might occur in thorough early debridement.

The primary utility of early minimal surgical debridement is through reducing the local vesicant dose and its spread by physically removing and lavaging the vesicant from affected soft tissue. It was demonstrated in this study that this can be accomplished by removing a minimum of cutaneous tissue, with retention of significant decreases in ulcer size (table 1) and accelerated ulcer healing (table 2) seen in the more extensive initial debridement previously described. Surgical interference with repetitive cycles of ongoing necrosis caused by release of vesicant from dead cells with subsequent binding to viable cells probably accounts for the marked decrease in the number of unhealed ulcers in the debrided groups compared to controls (table 2).

TABLE 3.—Comparison of doxorubicin ulcer size between single minimal surgical debridement groups and the redebridement of groups 2 wks following vesicant injections

	Minimal debridement site	Redebridement site
Time from injection to debridement (hrs)	Mean diameter of necrosis ± SD(mm)	Mean diameter of necrosis ± SD(mm)
1	4.7 ± 2.1	1.2 ± 1.3 ($P < 0.0005$)
24	6.1 ± 2.3	1.8 ± 3.0 ($P < 0.01$)
72	5.3 ± 2.9	2.0 ± 2.1 ($P < 0.05$)

Redebridement at a later date has been widely employed in other clinical situations to effectively treat lesions where clear margins of viability were initially not evident. This technique was applied in this study to certain groups to remove residual necrotic tissue and vesicant from the injection site with excellent effect (table 2).

The limitations of this study are obvious in that a rat model was employed, and caution is always advisable in applying conclusions from an animal study to human therapeutics. However, to simulate human clinical conditions, a concentration of doxorubicin was used that is administered in human chemotherapeutic regimens. Also, debridements were delayed (1, 24, or 72 hours) to simulate clinical delays in recognition or delays in preparing the patient for surgery.

Preliminary studies demonstrated the efficacy of immediate minimal debridement for doxorubicin, carmustine, dactinomycin, and mitomycin by producing significant reductions in ulcer size for all of these agents. Even though doxorubicin was the only vesicant evaluated in this study, it is probably safe to assume that the results reported regarding the efficacy of minimal debridement for doxorubicin extravasations may be applicable to other vesicant chemotherapeutic agents.

Although patients undergoing chemotherapy are often poor surgical candidates, early surgical debridement of significant vesicant extravasations in a sterile operating room with primary or secondary wound closure (possibly using skin grafting) in my hands has offered less risk in terms of infection, bleeding, and functional loss than attempting to manage established ulcers (2). Of eight extravasations referred to me, two with established ulcers had significant functional and cosmetic extremity impairment which persisted until their deaths, despite several surgical procedures. In contrast, three patients who had significant vesicant extravasations and underwent early debridement within 70 hours of the extravasation have excellent functional and cosmetic results at an average follow-up of 16 months. The remaining three patients were felt not to have significant extravasations and have excellent results after conservative therapy with an average follow-up of 5.9 months.

Larson's (7) experience with 50 human extravasations demonstrated that early surgical intervention is an excellent treatment modality for severe extravasations. However, it must be emphasized that not all extravasations require surgical intervention. Indeed, the majority do not. In Larson's series, only 24% of the extravasations required surgery. If pain and swelling persist after elevation and icing of the affected extremity over a 24-72-hour period of observation, this site is at risk for

necrosis, and surgical intervention should be strongly considered. The current study demonstrates that early surgical intervention is effective in reducing ulcer size without performing a radical debridement.

The prevention of these ulcers through early minimal surgical debridement may be in the best interest of these patients. One patient with a significant extravasation who has been treated surgically is presented to illustrate our approach to vesicant extravasations and report long-term follow-up.

CASE REPORT

A female (2 years and 6 months of age) who had undergone resection of a rhabdomyosarcoma from the left forearm sustained an extravasation of dactinomycin (0.005 mg/kg/day) to the dorsum of her right hand. Forty-eight hours after the extravasation, when called to evaluate the patient, her hand was markedly swollen dorsally and volarly, with multiple 1-2-mm vesicles on the dorsal surface. There was fusiform swelling of the fingers with limited active and passive finger motion, which caused severe pain. She was taken to the operating room approximately 6 hours later, where two 3-cm longitudinal incisions were made on the dorsum of the hand between the index and long, and the ring and small finger metacarpals. Fat necrosis was encountered in the dorsal subcutaneous space. Interosseous fasciotomies were performed because of suspicion of interosseous compartment syndrome, and these muscles appeared viable. Following debridement of necrotic tissue, the wounds were thoroughly irrigated with Ringer's lactate solution, and dressed open. At delayed primary closure 4 days later, there was no evidence of further necrosis or infection. Original operative cultures were negative for aerobic and anaerobic organisms, fungus, and acid-fast bacilli. The patient was treated in a bulky hand dressing with elevation and had an uncomplicated postoperative course. At follow-up 21 months after surgery, her surgical wounds were well-healed and she had full hand function.

In this case of frank necrosis in the subcutaneous space, the wounds were dressed open to allow continued drainage of vesicant with the edema fluid. Leaving the wound open and performing a redebridement with delayed primary closure 4 days later allowed re-evaluation of the wound.

It could be argued that the patient may have had spontaneous resolution of this extravasation with comparable results to surgery with no treatment at all. However, this was unlikely, since the patient underwent periods of observation in which no improvement in her clinical status was noted, suggesting ongoing tissue damage. From data derived from animal studies, we felt there was a high probability of significant necrosis at the dosage and concentration of vesicant she had received. The site of involvement, the dorsum of the hand, was one at risk for significant long-term morbidity. Therefore, an early aggressive approach was taken to prevent vesicant-induced ulceration, which was successful.

Additional accumulation of cases is necessary to further define the role of early surgery in the treatment of vesicant extravasations. This case, however, illustrates an effective, reliable means of preventing vesicant-induced ulcers, which represents a promising new method for the treatment of clinically significant extravasations in humans.

REFERENCES

1. LOTH TS, and EVERSMANN WW, JR. Treatment methods for extravasations of chemotherapeutic agents: a comparative study. Orthop Transactions 8:128, 1984.
2. LINDER RM, UPTON J, and OSTEEN R. Management of extensive doxorubicin hydrochloride extravasation injuries. J Hand Surg 8:32-38, 1983.
3. BARR RD, and BELBECK LW. The role of sensitization in soft-tissue necrosis induced by extravasation of anti-cancer drugs. Pediatr Res 13(4, part 2):429, 1978.
4. DUVALL E, and BAUMANN B. An unusual accident during the administration of chemotherapy. Cancer News 3:305-306, 1980.
5. IGNOFFO RJ, and FRIEDMAN MA. Therapy of local toxicities caused by extravasation of cancer chemotherapeutic drugs. Cancer Treat Rev 7:17-27, 1980.
6. RUDOLPH R, SUZUKI M, and LUCE JK. Experimental skin necrosis produced by Adriamycin. Cancer Treat Rep 63:529-537, 1979.
7. LARSON DL. Treatment of tissue extravasation by antitumor agents. Cancer 49:1796-1799, 1982.

Phase II Evaluation of Doxorubicin Plus Cisplatin in Advanced Thyroid Cancer: A Southeastern Cancer Study Group Trial[1,2]

Stephen D. Williams,[3] Robert Birch,[4] and Lawrence H. Einhorn[3,*]

Twenty-two evaluable patients with all cell types of advanced thyroid cancer were treated with the combination of doxorubicin (60 mg/m^2) plus cisplatin (60 mg/m^2). There were two brief partial remissions (9.1%). There was considerable toxicity, with one drug-related death. Combination chemotherapy as given in this study was not effective for this patient population. [Cancer Treat Rep 70:405–407, 1986]

Carcinoma of the thyroid can be subdivided into groups of diseases with differing natural histories and responses to therapy. Well-differentiated thyroid cancer (papillary, follicular, and Hürthle cell) is an indolent disease, with good results from initial treatment. Medullary carcinoma has a somewhat less favorable prognosis. Anaplastic carcinoma (giant cell, small cell, and spindle cell) is an aggressive neoplasm with a rapidly fatal course in the vast majority of patients.

Due to the rarity of these diseases, there is a paucity of data regarding chemotherapy for advanced disease. It appears that the most active single agent is doxorubicin (ADR) (1,2). In initial studies at the M. D. Anderson Hospital and Tumor Institute (1), 30 patients were treated, of whom 11 achieved partial remission. Responses were seen in all cell types.

Another agent that appears to have some activity is cisplatin (3–5), although the number of treated patients is small and a precise estimate of true response rate is impossible.

Accordingly, the Southeastern Cancer Study Group chose to evaluate the combination of these two agents in advanced thyroid cancer. A phase II nonrandom design was used because of the rarity of this disease.

PATIENTS AND METHODS

This study was initiated in May 1978 and concluded in January 1984. Eligibility requirements included a histologically confirmed diagnosis of thyroid cancer unsuitable for local therapy. All cell types were eligible, but patients with well-differentiated thyroid cancer were either to have been unsuitable for radioactive iodine therapy or to have exhibited progressive disease with adequate dosages. Bidimensionally measurable disease was required. Patients with significant organic heart disease, serum creatinine > 2 mg/100 ml, or prior chemotherapy with either study drug were excluded. Informed consent was required from all patients in accordance with individual institution policy.

Treatment was initiated with ADR (60 mg/m^2) by iv bolus plus cisplatin (60 mg/m^2) on Day 1. Cisplatin was given with vigorous hydration but without the routine use of mannitol or diuretics. Subsequent doses were to be escalated to 75 mg/m^2 in the absence of significant hematologic toxicity for ADR and for cisplatin if the serum creatinine remained < 1.5 mg/100 ml. Courses were given every 28 days in the absence of significant myelosuppression. ADR dose was reduced by 20% for a granulocyte count nadir < 750/mm^3.

Chemotherapy was to be continued until disease progression was noted or until a maximum cumulative dose of ADR of 450 mg/m^2 was reached, at which time all therapy was stopped.

Complete remission (CR) was defined as the disappearance of all clinically and radiographically demonstrable tumor. Partial remission (PR) was defined as a 50% decrease in the sum of the products of the largest perpendicular diameters of all measurable disease. Response duration was dated from the time a response was observed until disease progression. Survival was measured from initiation of treatment.

[1]Received Apr 15, 1985; revised July 29, 1985; accepted Aug 2, 1985.

[2]Supported in part by Public Health Service grants CA-19657, CA-24456, CA-28171, CA-15584, CA-07961, CA-03177, CA-12223, CA-13237, CA-28169, CA-28143, CA-03013, CA-05641, and CA-20255 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. L. H. Einhorn is an American Cancer Society Clinical Professor of Oncology.

[3]Indiana University, Indianapolis.

[4]Southeastern Cancer Study Group and the University of Alabama, Birmingham.

Reprint requests to: Southeastern Cancer Study Group, Operations Office, LBW Tumor Institute, University Station, Birmingham, AL 35294.

RESULTS

Twenty-eight patients were entered from 14 member institutions. Of these, 22 were fully evaluable for response. The reasons for exclusion and patient population are described in table 1. All cell types were represented. Only three patients had received other chemotherapy prior to entry.

Two PRs were seen (9.1%; 95% confidence limits, 2%-30%). One of the responding patients had well-differentiated carcinoma (duration, 16 weeks); the other had an anaplastic tumor (duration, 18 weeks). Virtually all patients have died at the time of this analysis; the median survival was 47 weeks.

One excluded patient is of considerable interest. At age 68, she developed a rapidly enlarging neck mass. Biopsy was said to show anaplastic carcinoma. She received five courses of protocol chemotherapy with prompt tumor regression. Postchemotherapy radiation was given and she now has been continuously disease-free for 48 months. However, subsequent institutional pathology review done without knowledge of the clinical history concluded that this lesion was lymphoma.

Toxicity was considerable. All patients exhibited at least some toxic effects. Thirty percent of the patients had absolute granulocyte counts $< 1000/mm^3$ during treatment. Fifty-two percent had moderate or severe gastrointestinal toxicity.

Two patients had serum creatinines > 2.0 mg/100 ml. There was one drug-related death in a patient with poor performance status and rapidly progressive disseminated anaplastic carcinoma. He died of gram-negative sepsis while neutropenic.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	28
Ineligible	3
No measurable disease	2
Pathology error*	1
Inadequate data	1
Intercurrent illness	2
Evaluable for response	22
Median age in yrs (range): 58 (21-83)	
Sex	
Male	9
Female	13
Cell type	
Medullary	6
Adenocarcinoma	1
Anaplastic	7
Follicular	4
Follicular and papillary	2
Hurthle cell	1
Unknown	1
Prior therapy	
None	3
Surgery	6
Surgery + radiotherapy	2
Surgery + ^{131}I	5
Surgery + ^{131}I + radiotherapy	3
Surgery + chemotherapy ± ^{131}I and radiotherapy	3
Karnofsky performance status	
80-100	10
40-70	9
Not stated	3
Areas of involvement	
Thyroid ± regional involvement	6
Lung ± locoregional	13
Thyroid + bone	1
Thyroid + bone + CNS	1
Thyroid + bone + lung + CNS	1

*See text.

DISCUSSION

The results of this study were disappointing. Within the confidence limits of this study, there is no evidence that chemotherapy is of major effectiveness. Likewise, there is no evidence that the addition of cisplatin confers benefits to those expected from ADR alone.

These results conflict somewhat with a recent study of the Eastern Cooperative Oncology Group (6). They compared in a random fashion ADR (60 mg/m^2) versus the same plus cisplatin (40 mg/m^2). Seven of 41 patients (17%) responded to the single agent versus 11 of 41 (27%) to the combination. However, there were five CRs in the combination arm versus none in the single-agent arm. The cell types of the patients achieving CR were not stated. Three CRs lasted > 2 years, with two patients continuing in remission at the time of publication of this abstract. The investigators concluded that the combination offered patients a chance for long-term unmaintained remission.

Bukowski et al (7) treated 11 patients with the combination of ADR, bleomycin, vincristine, and melphalan. There were three brief PRs and one CR. The latter occurred in a patient with anaplastic carcinoma and was continuing at 60+ months at the time of publication. Others have anecdotally reported durable chemotherapy-induced CR in anaplastic carcinoma (8).

The findings in some of these studies of an occasional patient who experiences long CR are provocative. Our study would have given similar findings had not blinded pathology review been conducted with a change of diagnosis of this responder to lymphoma. It is conceivable that there is a small subset of these patients who are very responsive to combination chemotherapy. Likewise, it is also possible that at least some of the durable responses described in other studies occurred in patients who in reality had lymphoma, which on occasion can be confused with anaplastic carcinoma pathologically (9,10).

In summary, our small study failed to define a role for the combination chemotherapy regimen employed. Likewise, we believe that available data have also not defined a role for other combination chemotherapy regimens as

compared to single-agent ADR. Future studies should be done in large cooperative groups and preferably done in a random fashion. Central pathology review should be included in these studies.

REFERENCES

1. GOTTLIEB JA, and HILL CS. Chemotherapy of thyroid cancer with Adriamycin. N Engl J Med 290:193-197, 1974.
2. POSTER DS, BRUNO S, PENTA J, ET AL. Current status of chemotherapy in the treatment of advanced carcinoma of the thyroid gland. Cancer Clin Trials 4:301-307, 1981.
3. REYES J, and SHIMAOKA K. Chemotherapy of thyroid carcinoma. Proc Am Assoc Cancer Res and ASCO 16:229, 1975.
4. KAMALAKAR P, FREEMAN AI, HIGBY DJ, ET AL. Clinical response and toxicity with *cis*-dichlorodiammineplatinum(II) in children. Cancer Treat Rep 61:835-839, 1977.
5. HIGBY DJ, WALLACE HJ, ALBERT DJ, ET AL. Diamminodichloroplatinum: a phase I study showing responses in testicular and other tumors. Cancer 33:1219-1225, 1974.
6. SHIMAOKA K, SCHOENFELD DA, LERNER H, ET AL. A randomized trial of adriamycin versus adriamycin plus *cis*-diammine-dichloroplatinum in patients with thyroid carcinoma. Proc ASCO 2:168, 1983.
7. BUKOWSKI RM, BROWN L, WEICK JK, ET AL. Combination chemotherapy of metastatic thyroid cancer. Am J Clin Oncol 6:579-581, 1983.
8. WEISBERG A, LUONGO V, and BOCHETTO J. Giant cell carcinoma of the thyroid gland: long survival after combination chemotherapy. South Med J 74:638-639, 1981.
9. HEIMANN R, VANNINEUSE A, DESLOOVER C, ET AL. Malignant lymphomas and undifferentiated small cell carcinoma of the thyroid: a clinicopathological review in the light of the Kiel classification for malignant lymphomas. Histopathology 2:201-213, 1978.
10. RAYFIELD EJ, NISHIGOMA RH, and SISSON JC. Small cell tumors of the thyroid. Cancer 28:1023-1030, 1971.

Lack of Synergy Between Cisplatin and Cytarabine Against Ovarian Carcinoma In Vitro[1,2]

Stephen B. Howell* and Susan Gill[3]

Synergy has been reported for the combination of cisplatin and cytarabine in a murine model and in several human cell lines in vitro. Clinical trials are under way with this combination. However, we were unable to demonstrate any significant synergistic interaction between these two agents in two human ovarian carcinoma cell lines with either 4-hour or continuous concurrent exposure in vitro at drug concentration producing 1–2 logs of cell kill. Synergy between these two drugs may be limited to unique cell lines and tumors.
[Cancer Treat Rep 70:409–410, 1986]

Several groups have reported synergy between cisplatin and cytarabine both in the murine L1210 leukemia model (1) and against human cells in tissue culture (2). The most striking synergy has been observed in vitro with the human colon carcinoma cell line LoVo (3). This effect was greatest at high concentrations of cytarabine (4×10^{-3} M), levels which are well above those readily maintained in plasma by iv administration.

Recently we have demonstrated that cisplatin and cytarabine can be safely administered ip, and that this route resulted in 12-fold greater exposure for the peritoneal cavity than for the plasma in the case of cisplatin (4) and 300- to 1000-fold greater exposure for cytarabine (5). The cytarabine concentrations attained in the peritoneal cavity were well within the range reported to produce synergistic interaction with cisplatin. Based on these data, we developed a combination ip chemotherapy program employing large doses of cisplatin and cytarabine, and this regimen has demonstrated promising activity in early phase II trials. Nevertheless, previous in vitro studies had suggested marked variation in the degree of synergy between cisplatin and cytarabine in different tumor types, and we wished to evaluate synergy directly for human ovarian carcinoma.

MATERIALS AND METHODS

The human ovarian carcinoma 2008 and COLO 316 cell lines were originally established in culture directly from primary tumors (6,7). The cells were grown in log-phase cultures in RPMI 1640 medium containing 10% fetal calf serum (Gibco Inc, Grand Island, NY). Both types of cells form well-defined colonies when seeded sparsely on plastic tissue culture dishes in the absence of agar.

Cisplatin was donated by Bristol Laboratories Inc, Syracuse, NY, and cytarabine was purchased from the Upjohn Co, Kalamazoo, MI. High-concentration stocks of both drugs were prepared by dissolution in 0.9% sodium chloride, and aliquots were stored frozen at -20°C. Drug stability was confirmed by high-pressure liquid chromatography.

In the case of 4-hour drug exposure, the cells were treated with cisplatin, cytarabine, or various combinations of both agents in suspension, then washed twice in RPMI 1640 with 10% fetal calf serum prior to being plated in triplicate at 400 cells per dish in 60-mm tissue culture plates. In the case of continuous exposure, drugs were added directly to tissue culture dishes after plating of the cells. The cultures were incubated for 10–12 days; cell aggregates of > 40 cells were scored as colonies. The cloning efficiency of the 2008 cells averaged 30%, and that of the COLO 316 cells averaged 25%

Triplicate cultures were prepared for each data point, and each experiment was repeated at least three times. All experiments were internally controlled for the activity of each drug alone. Synergy was assessed by comparing the product of the surviving fraction following treatment with each drug alone to that observed following treatment with both drugs together. The correlation coefficient for all regression lines in all experiments was > 0.95.

[1]Received Jan 14, 1985; revised Aug 20, 1985; accepted Sept 6, 1985.
[2]Supported by Public Health Service grant CA-35309 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from Bristol Laboratories, Inc. This work was conducted in part by the Clayton Foundation for Research, California Division. S. B. Howell is a Clayton Foundation investigator.
[3]Department of Medicine and the Cancer Center, University of California, San Diego, La Jolla, CA.
* *Reprint requests to:* Stephen B. Howell, MD, Department of Medicine, T-012, University of California, San Diego, La Jolla, CA 92093.

RESULTS

Continuous Exposure

Cisplatin and cytarabine produced log-linear dose-response curves with both cell lines when drug exposure was continued for the full period of colony formation, and both cell lines had similar sensitivities. The concentration of cisplatin required to reduce survival to 37% of control (D_0) was 1.8×10^{-7} M for the 2008 cell line and 1.0×10^{-7} M for COLO 316. The concentration of cytarabine necessary to produce the same decrease in survival was 1.7×10^{-8} M for the 2008 cell line and 3.8×10^{-9} M for COLO 316.

Evaluation of synergy with continuous exposure was carried out using a cytarabine concentration that reduced survival by 25%–75% when applied alone (10^{-8} M for 2008 and 5×10^{-9} M for COLO 316). The presence of cytarabine in the culture had only a minimal effect on the cisplatin dose-response curve of either cell line. The mean (± SD) dose modification factor (ratio of the slopes) was 1.3 ± 0.5 for the 2008 cell line and 1.4 ± 0.3 for COLO 316, neither of which represents a statistically significant difference (t test for paired differences). Thus, we could show no synergy using continuous exposure to a low concentration of cytarabine.

Four-Hour Exposure

Cisplatin produced log-linear dose-response curves against both cell lines using a 4-hour period of drug exposure prior to plating. The D_0 for the 2008 cell line was 1.5×10^{-6} M and for COLO 316 was 2.2×10^{-6} M. Thus, to produce equivalent degrees of cell kill against the 2008 cell line, an eightfold higher concentration of cisplatin was required when a 4-hour exposure was used than when continuous exposure was utilized. In the case of COLO 316, a 22-fold higher concentration was required. Of particular note is the fact that the slope of the cisplatin dose-response curve also differed markedly for the two schedules. For the 2008 cells, the slope was 12-fold steeper with the continuous exposure, and for the COLO 136 cells it was 22-fold steeper.

Cytarabine alone produced essentially no killing of either type of ovarian carcinoma cell with a 4-hour exposure at concentrations of up to 10^{-3} M. At this concentration, survival of 2008 cells was 115% ± 24% of control, and survival of COLO 316 cells was 100% ± 7% of control. As with the continuous exposure, a 4-hour exposure to 10^{-3} M cytarabine caused little change in the toxicity of cisplatin. The mean dose (± SD) modification factor for the 2008 cells was 1.3 ± 0.1, and it was also 1.3 ± 0.1 for the COLO 316 cells. Neither of these represents a statistically significant change in the slope of the dose-response curve.

DISCUSSION

The suggestion that cytarabine is synergistic with cisplatin comes from in vivo studies of L1210 murine leukemia (1) and in vitro studies of human colon carcinoma (3) and lymphoma cell lines (2). The clinical trials suggested that the combination produces greater myelosuppression than would be expected from a purely additive effect of the two drugs. Nevertheless, under carefully defined in vitro conditions, we were unable to demonstrate this interaction for two human ovarian carcinoma cell lines using either continuous exposure to low concentrations of drugs or short (4-hour) exposure to high concentrations of drugs such as those that are achievable with ip instillation. It should be noted that these schedules are slightly different from those reported to produce synergy against LoVo cells (1-hour cytarabine exposure), but directly reflect the schedules which are being used clinically. Although we used a 4-hour and continuous rather than a 1-hour exposure period, previous studies with the LoVo cell system suggested that the schedule of exposure had relatively little effect on the ability to demonstrate the synergistic interaction (3). Both the experience with the LoVo cell line and the early results of clinical trials suggest that there is no requirement for the effect of one drug to be established before the beginning of exposure to the second agent.

REFERENCES

1. SCHABEL FM, JR, TRADER MW, LASTER WR, ET AL. *cis*-Dichlorodiammineplatinum(II): combination chemotherapy and cross-resistance studies with tumors in mice. Cancer Treat Rep 63:1459–1473, 1979.
2. DREWINKO B, GREEN C, and LOO TL. Combination chemotherapy in vitro with *cis*-dichlorodiammineplatinum(II). Cancer Treat Rep 60:1619–1625, 1976.
3. BERGERAT JP, DREWINKO B, CORRY P, ET AL. Synergistic lethal effect of *cis*-dichlorodiammineplatinum and 1-3-D-arabinofuranosylcytosine. Cancer Res 41:25–30, 1981.
4. HOWELL SB, PFEIFLE CL, WUNG WE, ET AL. Intraperitoneal cisplatin with systemic thiosulfate protection. Ann Intern Med 97:845–851, 1982.
5. KING ME, PFEIFLE CE, and HOWELL SB. Intraperitoneal cytosine arabinoside therapy in ovarian carcinoma. J Clin Oncol 2:662–669, 1984.
6. WOODS LK, MORGAN RT, QUINN LA, ET AL. Comparison of four new cell lines from patients with adenocarcinoma of the ovary. Cancer Res 39:4449–4459, 1979.
7. DISAIA PJ, SINKOVICS JG, RUTLEDGE FN, ET AL. Cell-mediated immunity to human malignant cells. Am J Obstet Gynecol 114:979–989, 1972.

Effect of 1-Methyl-5-sulfonamide-4-nitroimidazole and 1-Methyl-5-bromide-4-nitroimidazole on the Radiosensitivity of EMT6 Tumor Cells[1,2,3]

Sara Rockwell,* Carolyn G. Irvin, and Marianne Nierenburg[4,5]

The compounds 1-methyl-5-sulfonamide-4-nitroimidazole and 1-methyl-5-bromide-4-nitroimidazole are extremely effective radiosensitizers of hypoxic cells in vitro. Preincubation of hypoxic cells with these and related drugs produces greater sensitization than is obtained with short incubations or is expected from the electron affinities of the compounds. The experiments reported here were undertaken to determine whether these agents were effective in sensitizing the naturally occurring hypoxic cells of solid tumors in mice. Neither of the 4-nitroimidazoles produced significant radiosensitization of the hypoxic cells of EMT6 mouse mammary tumors in vivo with any of the single- or multiple-injection regimens tested.
[Cancer Treat Rep 70:411–413, 1986]

MATERIALS AND METHODS

The compounds 1-methyl-5-sulfonamide-4-nitroimidazole (MJL) and 1-methyl-5-bromide-4-nitroimidazole (MTR) were synthesized by Dr. James Parham, Walker Laboratories, Sloan-Kettering Institute, Rye, NY. MJL was synthesized previously and independently and referred to as NSC-38070. Misonidazole (Miso) was obtained from Hoffmann-La Roche. Drugs were dissolved in physiological saline.

The effect of MJL on EMT6 cells in vitro was examined using monolayers made hypoxic by gassing with 95% N_2/5% CO_2 for 1 hour before irradiation with graded doses of 250 kV x-rays (1,2). Cell survival was measured by colony formation (3). Unirradiated cultures were included in each experiment to detect and correct for cytotoxicity from drugs or hypoxia. Sensitizer enhancement ratios (SER) were calculated from full dose-response curves as the ratios of the radiation doses necessary to reduce survival to 10^{-2} with and without drug (4). The oxygen enhancement ratio (OER) was calculated analogously.

Toxicity was estimated using BALB/c KaRw mice 3 months of age. Mice were observed for 5 days, during which toxic reactions were documented. Radiosensitization was tested using solid 100-mm^3 intradermal EMT6-Rw tumors in the flanks of 2.5-month-old BALB/c KaRw mice (3). Animals were irradiated whole-body, without anesthesia, using 250 kV x-rays (2). Immediately after irradiation, the four to six mice in each group were killed, the tumors were removed, a single-cell suspension was prepared, and the tumor cells were counted and assayed for viability by testing their ability to form colonies in vitro (3).

RESULTS

MJL was an exceptionally effective radiosensitizer of hypoxic EMT6 cells in vitro. The SER for cells irradiated after a 60-minute preincubation with 10^{-4} M MJL was 2.2; this was significantly larger than the SERs for 10^{-4} M Miso (1.2) or 10^{-3} M Miso (1.6), and was not significantly different from the OER (2.8). The exceptional radiosensitization documented for MJL in hamster cells (4–8), therefore, occurs in EMT6 cells in vitro as well.

The maximally tolerated doses of MJL and MTR were approximately 0.1 and 0.2 mg/g, respectively. These compounds were much more toxic than Miso, which has an LD_{50} of 1.5 mg/g in BALB/c mice (2). The symptoms observed in treated mice resembled those seen with Miso (2).

The radiosensitizing effects of the MJL and MTR in vivo were tested with intervals of 5, 30, or 60 minutes between ip injection and irradiation (fig 1). Drugs were

[1]Received Mar 20, 1984; revised Sept 16, 1985; accepted Sept 27, 1985.

[2]Supported by Public Health Service grant CA-06519 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]This paper is dedicated to the memory of Dr. James Parham, whose interest in radiosensitizers led to the studies reported here.

[4]Department of Therapeutic Radiology, Yale School of Medicine, New Haven, CT.

[5]We thank Dr. James Parham for providing the 1-methyl-5-sulfonamide-4-nitroimidazole and 1-methyl-5-bromide-4-nitroimidazole and Eric Hall and Myles Astor for sharing their data on the compounds.

* *Reprint requests to:* Sara Rockwell, PhD, Department of Therapeutic Radiology, Yale School of Medicine, Hunter Radiation Therapy, PO Box 3333, 333 Cedar St, New Haven, CT 06510.

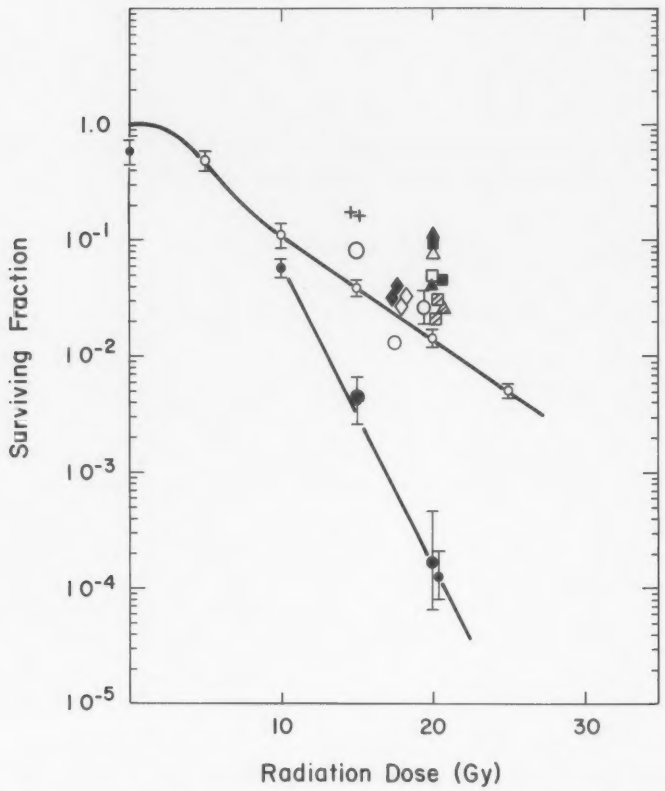

FIGURE 1.—Survival of cells from EMT6 tumors treated in vivo. Small circles, lines = composite curves for irradiation alone (○) and irradiation given 1 hr after 1 mg/g of Miso (●) (*from* ref 1). Large circles = survivals after irradiation alone (○) or irradiation plus Miso (●) from the experiments reported here. SEMs are shown where larger than points. For MJL and MTR, individual surviving fraction determinations are shown. Open symbols = 0.2 mg/g of MTR; closed symbols = 0.1 mg/g of MJL; and hatched symbols = 0.01 mg/g of MJL. Drugs were injected ip 5 (diamonds), 30 (squares), or 60 (triangles) mins before irradiation. Crosses = 5 ip injections of 0.02 mg/g of MJL, 60, 45, 30, 15, and ~1 min before irradiation.

tested at the maximally tolerated doses; MJL was also tested at a dose of 0.01 mg/g because of the high SERs obtained in vitro at low MJL concentrations (4,6,7). Experiments testing MJL and MTR also included untreated controls, groups treated only with irradiation, and groups treated with 1 mg/g of Miso (ip, 1 hour before irradiation). Miso was a very effective radiosensitizer of EMT6 tumors, reducing the D_0 of the tail of the survival curve by a factor of 2.1 (1). In contrast, neither MJL nor MTR sensitized the tumors on any regimen tested.

Iv injection of MJL or MTR 5 minutes before the start of irradiation likewise failed to alter the radiosensitivity of the tumor cells. In this study (performed using the protocol described above but with another x-ray machine having different dosimetric characteristics), the surviving fraction of cells from tumors treated with 20 Gy of x-rays was 0.0050 ± 0.0005. The surviving fractions for tumors irradiated after treatment with 0.1 mg/g of MTR (0.0042 ± 0.005) or 0.2 mg/g of MJL (0.0060 ± 0.005) were not significantly different from the survivals after irradiation alone.

It was hypothesized that multiple injections of these agents might be preferable to single injections, as administration of several small doses might result in prolonged exposure of the tumor cells to the drug and enhanced radiosensitization, similar to that observed in vitro after preincubation of cells with MJL (4,6,7). To test this possibility, tumor-bearing mice were treated with five injections of MJL (0.02 mg/g/injection), 60, 45, 30, 15, and ~1 minute before irradiation. This regimen did not sensitize the tumor cells to irradiation (fig 1). The host toxicity of this regimen was similar to that of a single injection of 0.1 mg/g of MJL; this severe toxicity precluded tests of multiple-treatment regimens using higher drug doses.

DISCUSSION

The naturally occurring hypoxic cells of solid EMT6 tumors in vivo were not sensitized by any of the single-treatment MJL or MTR regimens or by the five-injection MJL regimen. This is in contrast to findings with these and similar 4-nitroimidazoles in vitro (4-9). After short contact times (~5 mins) in vitro, MJL has radiosensitizing activity similar to that of Miso, as would be expected on the basis of its electron affinity; as the duration of preincubation increases, the radiosensitization increases dramatically, producing SERs far in excess of those obtained with Miso under similar conditions (4,6,7). However, this does not reflect oxygen-mimetic radiosensitization, but results from depletion of intracellular nonprotein sulfhydryl compounds during preincubation; removal of these endogenous radioprotectors sensitizes the cells (4,6,7). Our limited in vitro studies confirmed that MJL is highly effective in sensitizing EMT6 cells in vitro, indicating that differences between Chinese hamster cells (4-8) and our EMT6 mouse mammary tumor cells are not responsible for the discrepancy between our findings in vivo and published findings in vitro.

The failure of MJL and MTR to produce radiosensitization in vivo may reflect several factors. First, the drugs were relatively toxic to mice; as a result, the doses tested were limited to about 10% of the dose possible with Miso. As 4-nitroimidazoles similar to those tested here are extremely reactive and are inactivated rapidly in vivo (5,8,9), it is also possible that the drugs never reach the hypoxic tumor cells in the high concentrations necessary for oxygen-mimetic radiosensitization or for the long times necessary to produce radiosensitization via depletion of intracellular nonprotein sulfhydryl compounds. It is possible that different schedules, involving infusions or a different schedule of multiple injections, might prove efficacious; data on the pharmacokinetics of the drugs would be necessary to assess whether efficacious treatment regimens could be devised.

In summary, although MJL and MTR are extremely useful as lead compounds for examining the mechanisms of action of hypoxic cell radiosensitizers in vitro, they did not exhibit sensitizing activity when tested using solid tumors in mice and treatment regimens employing either single injections of a maximally tolerated dose or multiple injections (five injections, 15 mins apart) to the same dose and same level of toxicity.

REFERENCES

1. ROCKWELL S. Hypoxic cells as targets for cancer chemotherapy. *In* The Development of Target-Oriented Anticancer Drugs. New York, Raven Press, 1983, pp 157-172.
2. ROCKWELL S, MROCZKOWSKI Z, and RUPP WD. Evaluation of 2-amino-5-nitrothiazole as a hypoxic cell radiosensitizer. Radiat Res 90:575-585, 1982.
3. ROCKWELL S. In vivo-in vitro tumor systems: new models for studying the response of tumors to therapy. Lab Anim Sci 27:831-851, 1977.
4. HALL EJ, ASTOR M, BIAGLOW J, ET AL. The enhanced sensitivity of mammalian cells to killing by x-rays after prolonged exposure to several nitroimidazoles. Int J Radiat Oncol Biol Phys 8:447-451, 1982.
5. ADAMS GE, AHMED I, FIELDEN EM, ET AL. The development of some nitroimidazoles as hypoxic cell sensitizers. *In* Radiation Sensitizers. New York, Masson Press, 1980, pp 33-38.
6. ASTOR M, HALL EJ, MARTIN J, ET AL. Radiosensitizing and cytotoxic properties of ortho-substituted 4- and 5-nitroimidazoles: role of NPSH reactivity. Int J Radiat Oncol Biol Phys 8:409-413, 1982.
7. BIAGLOW JE, VARNES ME, ASTOR M, ET AL. Non-protein thiols and cellular response to drugs and radiation. Int J Radiat Oncol Biol Phys 8:719-723, 1982.
8. MA H, HARDY CR, and O'NEILL P. Formation of halide-ions on one-electron reduction of halogenated nitroimidazoles in aqueous solution. A radiolytic study. Int J Radiat Biol 41:151-160, 1982.
9. WARDMAN P. The kinetics of the reaction of "anomalous" 4-nitroimidazole radiosensitizers with thiols. Int J Radiat Biol 41:231-235, 1982.

Cisplatin in Patients With Gastric Cancer: A Cancer and Leukemia Group B Phase II Study[1,2]

Michael C. Perry,[3,*] Mark R. Green,[4] Rosemarie Mick,[5] and Philip Schein[6,7]

In spite of a decline in recent incidence, gastric cancer continues to be the sixth most common cause of cancer deaths in the United States, with an overall 5-year survival rate still in the 5%–15% range (1). Several combination drug programs have been shown to be more effective than single agents, but new agents with activity are needed (2). Cisplatin is a metal complex that has demonstrated clinical activity against various neoplasms, including testis, ovary, lung, and possibly brain (3). Pilot studies at Georgetown University demonstrated sufficient patient tolerance, but the activity of cisplatin in gastrointestinal malignancy was essentially unknown at the time this study was undertaken. In 1980, Cancer and Leukemia Group B (CALGB) sought to investigate the activity of cisplatin in patients with previously treated gastric adenocarcinoma in a phase II study.

MATERIALS AND METHODS

To be eligible for the study, patients had to have histologically confirmed, measurable unresectable, or metastatic adenocarcinoma of the stomach. Patients had to have a performance score of 3 or better (CALGB criteria), a minimal life expectancy of at least 2 months, and, at most, treatment with one prior chemotherapy regimen. In addition, patients had to have a creatinine clearance of at least 60 ml/min.

An initial cisplatin dose of 75 mg/m^2 was administered iv every 3 weeks. An escalation to 100 mg/m^2 was allowed after the first dose if adequate tolerance was observed. Treatment was repeated until there was evidence of disease progression.

Complete response was defined as 100% disappearance of all measurable disease and complete disappearance of all signs, symptoms, and biochemical changes related to the tumor. Partial response was defined as a reduction of at least 50% in the sum of the products of all measurable lesions. Progressive disease was defined as an increase in the product of two perpendicular diameters by $\geq$ 25%.

Fourteen patients were entered in the study, seven females and seven males (table 1). The age range was from 38 to 77 years, with an average of 55.7 years. Eight of the 14 patients (62%) had a performance score of 1, two (15%) had a performance score of 2, and three (23%) had a performance score of 3. Eleven of the patients (79%) had undergone surgery and 12 (86%) had received prior combination chemotherapy, most with 5-FU, doxorubicin, and mitomycin (FAM) (2). None of the patients had received prior radiation therapy or immunotherapy.

RESULTS

One of the 14 patients left the country and no response information was available. Of the remaining 13 patients, there was one partial response (nodal disease), one complete response (skin disease), and two improvements short of partial response (other visceral disease). Nine patients demonstrated progressive disease. Median survival time for the 14 patients was 3.9 months, with a minimum of 0.8 months and a maximum of 12.5 months. The patient who survived 0.8 months died of progressive disease. The two responders experienced response durations of 4 and 5 months. Four patients had dose escalations; one of these was the patient with a complete response. No correlation between initial response to FAM chemotherapy and response to cisplatin was observed.

Moderate nausea and vomiting was seen in seven patients and one patient experienced severe nausea and

[1]Received Mar 6, 1985; revised June 21, 1985; accepted July 18, 1985.

[2]This study was conducted by Cancer and Leukemia Group B (*Emil Frei, III*, chairman, CA-31946) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: Columbia University, New York, NY (CA-12011); Dana-Farber Cancer Institute, Boston, MA (CA-32291); Finsen Institute, Copenhagen, Denmark; Massachusetts General Hospital, Boston (CA-12449); New York Hospital, New York (CA-07986); University of California at San Diego (CA-11789); University of Minnesota, Minneapolis (CA-16450); University of West Virginia, Charleston (CA-28652); Walter Reed Army Medical Center, Washington, DC (CA-26806); and Oschner Clinic, New Orleans, LA (CA-35272).

[3]Department of Medicine, University of Missouri-Columbia (CA-12046).

[4]University of California at San Diego (CA-11789).

[5]CALGB Statistical Office, Harvard School of Public Health, Boston, MA (CA-33601).

[6]Georgetown University Hospital, Washington, DC.

[7]*Present address:* Clinical Research and Development, Smith Kline and French Laboratories, Philadelphia, PA (CA-21083).

[*]*Reprint requests to:* Michael C. Perry, MD, Department of Medicine, University of Missouri-Columbia, MA406C Health Sciences Center, Columbia, MO 65212.

TABLE 1.—Patient clinical characteristics

Characteristic	No. of patients (%)
Sex	
Male	7 (50)
Female	7 (50)
Age in yrs	
< 39	1 (6)
40-49	4 (29)
50-59	3 (21)
60-69	4 (29)
70+	2 (14)
Performance status (CALGB)	
1	8 (62)
2	2 (15)
3	3 (23)
Prior treatments	
Yes	
Surgery	11 (79)
Combination chemotherapy	12 (86)
No	
Surgery	3 (21)
Combination chemotherapy	2 (14)
Sites of measurable disease*	
Nodes	4
Liver	1
Skin	1
Other viscera	12

*To be followed for response.

vomiting. Three patients developed severe thrombocytopenia and one had severe renal toxicity manifested by a decrease in creatinine clearance.

DISCUSSION

In this group of previously treated patients, cisplatin produced one complete and one partial response for a 16% response rate. Toxicity was tolerable. These results are comparable to those of other groups who found response rates of 20%-33% using a variety of doses and schedules (4-7). Since cisplatin's mechanism of action and toxicity are distinct from most other agents used in the treatment of gastric cancer, this agent may be of use in the design of new combination chemotherapy programs for gastric cancer.

REFERENCES

1. CARTER SK, and COMIS RL. Gastric cancer: current status of treatment. J Natl Cancer Inst 58:567-578, 1977.
2. MACDONALD JS, WOOLLEY PY, SMYTHE T, ET AL. 5-Fluorouracil, adriamycin, and mitomycin-C (FAM) combination chemotherapy in the treatment of advanced gastric cancer. Cancer 44:42-47, 1979.
3. ROZENCWEIG M, VON HOFF DD, SLAVIK M, ET AL. *cis*-Diamminedichloroplatinum (II): a new anti-cancer drug. Ann Intern Med 86:803-812, 1977.
4. LACAVE AJ, IZARZUGAZA I, ANTON APARICIO LM, ET AL. Phase II clinical trial: *cis*-dichlorodiammine platinum in gastric cancer. Am J Clin Oncol 6:35-38, 1983.
5. BEER M, COCCONI G, VARINI M, ET AL. A phase II trial of *cis*-diamminedichloroplatinum (DDP) in patients with stomach cancer. *In* Proceedings of the UICC Conference on Clinical Oncology, Lausanne, Switzerland, 1984, p 13.
6. VOGL SE, CAMACHO FJ, ENGSTROM PF, ET AL. Phase II trial of cisplatin in advanced gastric cancer. Cancer Treat Rep 68:1497-1498, 1984.
7. LEICHMAN L, MCDONALD B, DINDOGRU A, ET AL. Cisplatin: an active drug in the treatment of disseminated gastric cancer. Cancer 53:18-22, 1984.

Phase II Trial of Aminothiadiazole in Advanced Non-Small Cell Lung Cancer[1,2]

D. H. Shevrin,[3,*] W. P. McGuire,[3] V. Lanzotti,[4] S. Krauss,[5] R. Blough,[6] M. Cobleigh,[3] C. Johnson,[6] T. Lad,[7] and L. Kukla[7]

Aminothiadiazole (ATDA) is one of a series of 2-substituted-1,3,4-thiadiazoles, compounds which increase uric acid production that is not related to tissue destruction, but is due to an increase in uric acid biosynthesis de novo (1). Its mechanism of action is suggested to be similar to tiazofurin, ie, inhibition of inosine-5[1] phosphate (IMP) dehydrogenase, which in turn prevents the synthesis of guanosine monophosphate (2). ATDA has been selected for clinical trials because it has significant activity against several animal tumors. Phase I human studies found stomatitis to be the dose-limiting toxicity. Hyperuricemia developed in all patients, but was effectively inhibited by allopurinol administration (3). A phase II study of ATDA in advanced non-small cell lung cancer is the subject of this report.

PATIENTS AND METHODS

Patients with histologically confirmed non-small cell lung cancer, measurable tumor, Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2, and no evidence of hyperuricemia or gout received ATDA every week. ATDA was administered iv at an initial dose of 125 mg/m^2 in 150 ml of 5% dextrose in water over 30 minutes. Escalation to a maximum dose of 175 mg/m^2 was permitted. Dose de-escalation was carried out for stomatitis and hematologic toxicity (based on ECOG criteria). Allopurinol (300 mg) was administered orally on a daily basis starting the day before ATDA administration and continuing for the following 3 days. Patients were stratified by prior therapy: prior chemotherapy and/or radiation therapy versus no prior therapy. Standard ECOG response criteria were employed.

RESULTS

A total of 31 patients (table 1) were entered in the study, and 26 received an adequate trial of drug (at least two courses). There were two early deaths, one patient developed exfoliative dermatitis secondary to allopurinol, and one patient dropped out due to drug toxicity (stomatitis). One patient had inadequate tumor measurements to assess response. There were no objective responses. Eleven patients had stable disease lasting from 42 to 175 days. The median number of courses of ATDA was 6.5 for patients with no prior treatment and 4.5 for previously treated patients. Fourteen of 18 previously untreated patients were escalated to 150 mg/m^2 and seven of these were escalated to 175 mg/m^2.

TABLE 1.—Patient characteristics and results*

Characteristic	No prior therapy	Prior therapy
Entered	21	10
Evaluable	18	8
Median age in yrs (range)	61 (35-75)	59 (43-65)
Sex		
Male	18 (86)	7 (70)
Female	3 (14)	3 (30)
Performance status		
0	2 (10)	2 (20)
1	12 (57)	5 (50)
2	7 (33)	3 (30)
Histologic type		
Squamous cell carcinoma	11 (52)	7 (70)
Adenocarcinoma	5 (24)	2 (20)
Large cell carcinoma	5 (24)	1 (10)
Median No. of ATDA courses (range)	6.5 (2-38)	4.5 (2-19)
Response		
Partial	0	0
Stable	8 (44)	3 (38)
Disease progression	10 (56)	5 (62)

* Unless otherwise specified, values = No. of patients (%).

[1]Received July 20, 1984; revised July 15, 1985; accepted July 19, 1985.
[2]Supported in part by Public Health Service grant CA-21742 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3]Section of Medical Oncology, Department of Medicine, University of Illinois at Chicago.
[4]Section of Medical Oncology, Department of Medicine, Southern Illinois University, Springfield.
[5]Section of Medical Oncology, Weiss Memorial Hospital, Chicago, IL.
[6]Illinois Comprehensive Cancer Center, Chicago.
[7]West Side Veterans Hospital, Chicago IL.
* *Reprint requests to:* D. H. Shevrin, MD, Department of Medicine, University of Illinois, PO Box 6998, Chicago, IL 60680.

Toxicity

Thirty-one patients were evaluable for toxicity. Eleven patients developed mild or moderate stomatitis, which resolved with dose de-escalation. Eleven patients developed nausea and vomiting, which was mild or moderate in nine and severe in two. Marked anorexia was seen in four patients. Leukopenia and thrombocytopenia were not observed.

DISCUSSION

This study included patients with a good performance status; 18 of 26 evaluable patients were previously untreated. Although toxicity was seen, no major responses were observed. We conclude that ATDA at this dose and schedule is ineffective in the treatment of advanced non-small cell lung cancer.

REFERENCES

1. KRAKOFF IH, and BALIS ME. Studies of the uricogenic effect of 2-substituted thiadiazoles in man. J Clin Invest 38:907-915, 1959.
2. NELSON JA, ROSE LM, and BENNETT LL. Mechanism of action of 2-amino-1, 3, 4-thiadiazole (NSC 4728). Cancer Res 37:182-187, 1977.
3. MYERS CF, NEWMAN RA, HACKER MP, ET AL. Clinical studies of 2-amino-1, 3, 4-thiadiazole (ATDA). Proc Am Assoc Cancer Res 23:136, 1982.

Phase II Study of Sequential Methotrexate and 5-FU Combination in the Treatment of Advanced Colorectal Cancer[1]

Luciano Canobbio, Maria Teresa Nobile,* Andrea Ardizzoni, Rita Tatarek, and Riccardo Rosso[2]

Recently it has been shown that 5-FU cytotoxic activity might be enhanced by modulation of its metabolism in tumor cells. Laboratory findings in experimental tumor systems suggested that methotrexate (MTX) pretreatment of tumor cells followed by 5-FU may result in synergistic cell kill (1,2). Encouraging results have been obtained in preliminary clinical trials (4). These data led us to initiate a phase II study in advanced colorectal cancer patients, mostly pretreated with 5-FU, to evaluate the capability of the sequential MTX-5-FU combination to overcome 5-FU tumor resistance.

PATIENTS AND METHODS

Twenty consecutive patients with histologically proven metastatic colorectal carcinoma entered the study (table 1). All patients had measurable disease, an Eastern Cooperative Oncology Group performance score ≤ 2, an expected survival of at least 8 weeks, a wbc count $\geq 4000/mm^3$, a platelet count $\geq 125,000/mm^3$, serum bilirubin < 2.0 mg/dl, and a creatinine clearance ≥ 60 ml/m^2. Patients were treated with 200 mg/m^2 of MTX iv in a 1-hour infusion, followed 24 hours later by 600 mg/m^2 of 5-FU iv bolus. Twenty-four hours after the infusion of MTX, leucovorin rescue (10 mg/m^2 orally) was given every 6 hours for four times. Treatment was repeated every 14 days and continued until disease progression occurred or unless toxicity developed. All patients who received at least two courses were considered evaluable for response and toxicity according to World Health Organization criteria (3).

TABLE 1.—Patient and treatment characteristics

Characteristic	No. of patients
Total	20
Median age in yrs (range): 60 (35-72)	
Median performance status (range): 1 (0-2)	
Prior chemotherapy (including 5-FU)	16
Dominant site of disease	
Liver	13
Lung	6
Pelvis	1
Response	
Partial	1
Stable disease	11
Disease progression	7
Mean No. of courses (range): 4.6 (2-9)	
Toxicity	
Leukopenia (grade 1)	4
Anemia (grade 2)	1
Mucositis	
Grade 1-2	7
Grade 3	2
Nausea and vomiting (grade 3)	4
Alopecia (grade 2)	4
Conjunctivitis	3
Diarrhea	2

RESULTS

Nineteen of the 20 patients were evaluable for response. One patient was excluded because of early death unrelated to drug toxicity. One partial response documented by computerized tomographic scan was observed after three courses in one pretreated patient bearing hepatic metastatic lesions; response and survival lasted 12 and 30 weeks, respectively. In another patient, a normalization of hepatic function and marker (CEA) was noted, but without a > 50% reduction of the measurable lesions, and he was included in disease stabilization lasting 12 weeks. Disease stabilization occurred in 11 patients (57.9%); the median duration was 12 weeks (range, 8-28). The remaining seven patients (36.8%) had progressive disease. Median survival was 26 weeks (range, 12-56) for patients with stable disease and 10 weeks (range, 4-16) for patients with progressive disease.

Chemotherapy was well-tolerated. Myelosuppression was mild: four patients developed leukopenia (grade 1) and only one had anemia (grade 2). Mucositis was severe (grade 3) in two patients and minor (grade 1-2) in seven. Three patients developed conjunctivitis, three had mild nausea, four had severe vomiting (grade 3), and two developed diarrhea. Alopecia (grade 2) was observed in four patients.

[1]Received Mar 8, 1985; revised June 24, 1985; accepted July 8, 1985.
[2]Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy.

* *Reprint requests to:* Maria Teresa Nobile, MD, Istituto Nazionale per la Ricerca sul Cancro, V. le Benedetto XV, 10, 16132, Genova, Italy.

DISCUSSION

The disappointing response rate observed using this sequential MTX-5-FU combination in patients with previously treated colorectal carcinoma is partially in contrast to the results of other authors (4), although negative results have also been reported (5). Clinical and experimental data indicate that the MTX-5-FU sequential synergism is clearly a well-defined time- and dose-dependent phenomenon. Although this synergism can be evidenced under laboratory conditions, it can be difficult to achieve in the poorly understood and complex biochemical events that occur in humans. However, the fact that the MTX-5-FU combination is well-tolerated supports further trials focusing on the doses and time intervals in a variety of tumor types sensitive to this drug combination.

REFERENCES

1. BENZ C, TILLIS T, TATTELMAN E, ET AL. Optimal scheduling of methotrexate and 5-fluorouracil in human breast cancer. Cancer Res 42:2081-2086, 1982.
2. BERTINO JR, SAWICKI WL, LUIDQUIST CA, ET AL. Schedule-dependent antitumor effects of methotrexate and 5-fluorouracil. Cancer Res 37:327-328, 1977.
3. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207-214, 1981.
4. BRUCKNER HN, and COHEN J. MTX/5-FU trials in gastrointestinal and other cancers. Semin Oncol 10(suppl 2):32-39, 1983.
5. KAYE SB, SANGSTER G, HUTCHEON A, ET AL. Sequential methotrexate plus 5-FU in advanced breast and colorectal cancers: a phase II study. Cancer Treat Rep 68:547-548, 1984.

Phase II Trial of Carboplatin in Non-Small Cell Lung Cancer[1]

Ian N. Olver,[2] Ross C. Donehower,[3] David A. Van Echo,[2] David S. Ettinger,[3] and Joseph Aisner[2,*]

Cisplatin alone and particularly in combination with other drugs has demonstrated antitumor activity against several human malignancies including non-small cell lung cancer (1), but dose-limiting side effects have been nephrotoxicity and severe nausea and vomiting.

Carboplatin is a cisplatin analog which in preclinical testing retained antitumor activity (2) but was less nephrotoxic and emetogenic in both preclinical (3) and phase I clinical studies (4,5). It was, therefore, appropriate to conduct a phase II study of carboplatin in non-small cell lung carcinoma.

METHODS

Eligibility criteria included: measurable, histologically documented, unresectable or metastatic non-small cell lung cancer; performance status of at least 2 [Eastern Cooperative Oncology Group (ECOG) scale]; life expectancy of at least 12 weeks; wbc count > 3500/μl; platelet count > 100,000/μl; BUN ⩽ 25 mg/dl or creatinine clearance ⩾ 60 ml/minute; and bilirubin ⩽ 1.5 mg/dl with SGOT ⩽ 40 mU. Evaluable, nonmeasurable disease was excluded.

Carboplatin was given at a dose of 80 mg/m^2/day by iv bolus (infused over 15 minutes) for 5 days every 4 weeks in patients with no previous treatment or only limited-field radiation therapy and 60 mg/m^2/day to patients who had received either previous chemotherapy or extensive irradiation. Subsequent doses were modified by 25% if the wbc count was < 3500/μl or the platelet count was < 100,000/μl. Standard definitions of response were used.

RESULTS

Of 43 patients entered in the study (table 1), 41 were evaluable for response. One patient died early due to cerebral metastases and another patient, who achieved a partial response after two courses, was found, on review of the histology, to have a small cell carcinoma.

Although most patients had good performance status and no prior chemotherapy, no complete or partial re-

TABLE 1.—Patient characteristics and study results

Characteristic	No. of patients
Total entered	43
Evaluable	
For response	41
For toxicity	40
Median age in yrs (range): 62 (37-79)	
Sex	
Male	33
Female	10
Performance status (ECOG)	
0	13
1	22
2	8
Histology	
Adenocarcinoma	23
Squamous cell carcinoma	13
Large cell undifferentiated carcinoma	6
Small cell carcinoma *	1
Previous treatment	
Radiation therapy	18
Chemotherapy	2
Interferon	1
None	17
No. of cycles	
Total: 112	
Median (range): 2 (1-9)	
Toxicity (ECOG scale)	
Hematologic	
Thrombocytopenia grade—	
1	10
2	7
3	5
4	1
Leukopenia grade—	
1	9
2	13
3	5
4	0
Nausea and vomiting grade—	
1	9
2	9
3	1
Renal (mild)	1
Neurological	0

* Ineligible after review of histology.

[1]Received Mar 13, 1985; revised June 19, 1985; accepted July 8, 1985.

[2]University of Maryland Cancer Center, Baltimore.
[3]Johns Hopkins Oncology Center, Baltimore, MD.

* *Reprint requests to:* Joseph Aisner, MD, University of Maryland Cancer Center, 22 S Greene St, Baltimore, MD 21201.

sponses were seen. Of 29 patients who had stable disease for at least 1 month, one remained stable for 8 months, two for 5 months, one for 4 months, five for 3 months, and six for 2 months.

Forty patients were evaluable for toxicity. One patient died early and two had incomplete records. The most significant toxic effect was hematologic (table 1), with six patients having grade 3–4 thrombocytopenia, while five had grade 3 leukopenia (ECOG scale), both with a median time to nadir of 22 days. Renal function remained stable in all patients but one, whose serum creatinine rose from 1.0 to 2.7 mg/100 ml after three courses with no obvious cause and none subsequently found at autopsy. No neurological toxic effects were observed.

DISCUSSION

Phase I studies demonstrated that a larger dose of carboplatin per course could be tolerated using a fractionated rather than single-dose schedule (4,5). Carboplatin was well-tolerated, but at the dose and schedule given in this study it has shown little efficacy in non-small cell lung cancer. This trial demonstrates that the drug has a response rate of $< 10\%$ with $> 95\%$ confidence. Further studies with this agent as a single drug given in bolus fashion in non-small cell lung cancer do not seem warranted. Other schedules such as prolonged infusions are currently under study to test the correlation of the short circulating half-life. In addition, the potential for synergy with other agents remains to be explored.

REFERENCES

1. ROSSOFF AH, BEARDEN JD, III, COLTMAN CA, JR, ET AL. Phase II evaluation of *cis*-diamminedichloroplatinum (II) in lung cancer. Cancer Treat Rep 60:1679–1680, 1976.
2. Clinical Brochure: CBDCA (NSC-241240). Bethesda, MD, National Cancer Institute, Sept 1981.
3. HARRAP KR, JONES M, WILKENSON, ET AL. Antitumor, toxic and biochemical properties of cisplatinum and eight other platinum analogues. *In* Cisplatin: Current Status and New Developments (Prestayko A, Crooke ST, and Carter SK, eds). New York, Academic Press, Inc, 1980, pp 193–212.
4. ROZENCWEIG M, NICAISE C, BEER M, ET AL. Phase I study of carboplatin given on a five-day intravenous schedule. J Clin Oncol 1:621–626, 1983.
5. CALVERT AH, HARLAND SJ, NEWELL DR, ET AL. Early clinical studies with *cis*-diammine-1,1-cyclobutane, dicarboxylate platinum II. Cancer Chemother Pharmacol 9:140–147, 1982.

Phase II Trial of Bisantrene in Previously Treated Patients With Ovarian Carcinoma: A Southwest Oncology Group Study[1,2]

John D. Cowan,[3] Earl A. Surwit,[4] David S. Alberts,[4] John G. Boutselis,[5] and Barbara A. Neilan[6,*]

Bisantrene is a new anthracene compound which, in preclinical screening, showed a high degree of activity in a number of animal tumor systems (1). In addition, activity has been identified for the agent against fresh human ovarian cancers in the human tumor cloning assay (2). Phase I study of bisantrene, using a single dose on an every-3-week schedule, showed that its dose-limiting toxicity is leukopenia (3). Based on these data, the present phase II study was performed to determine the antitumor effect of bisantrene in patients with advanced ovarian carcinoma.

PATIENTS AND METHODS

Eligibility criteria included the following: histologically confirmed metastatic ovarian carcinoma in previously treated patients; informed consent; an evaluable lesion; performance status of ≥ 3 (Southwest Oncology Group criteria); no significant cardiac disease; prior treatment with < 400 mg/m^2 of doxorubicin; no concomitant antitumor therapy; a wbc count $> 4000/\mu l$; a platelet count $> 125{,}000/\mu l$; serum creatinine ≤ 2 mg/dl; and a bilirubin of < 2 mg/dl. Patient characteristics are shown in table 1.

Based on a phase I study (3), bisantrene was administered iv at an initial dose of 260 mg/m^2 (208 mg/m^2 in poor-risk patients) over at least 120 minutes in 1000 ml of 5% dextrose in water. Use of a central venous catheter was encouraged because of drug infusion-associated phlebitis. Patients were pretreated with 50 mg of diphenylhydramine hydrochloride im and 100 mg of hydrocortisone sodium succinate iv in order to decrease the risk of anaphylaxis (3). The dose was repeated every 3 weeks, if the wbc count was $> 3500/\mu l$ and the platelet count was $> 125{,}000/\mu l$. Dose modifications were encouraged and based on the nadir wbc and platelet counts. If the nadir wbc count was $> 4000/\mu l$ and the platelet count was $> 100{,}000/\mu l$, the dose was increased by 10%. If the nadir wbc count was $< 2000/\mu l$ and/or the platelet count was $< 50{,}000/\mu l$, the dose was decreased by 20%. Standard objective response criteria were used.

RESULTS

All of the 36 patients entered on the study were eligible for toxicity evaluation, with 30 of these being evaluable for response. One patient died of progressive tumor on Day 16 after the first course of therapy; three patients had no evaluable lesion; one received concomitant radiation therapy; and one received the wrong drug dosage.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 58 (29-79)	
Median performance status (range): 1 (0-3)	
Good-risk/poor-risk	21/15
Prior therapy	
Chemotherapy	36
Doxorubicin	34
Median No. of regimens (range): 2 (1-5)	
Median No. of agents (range): 4 (3-7)	
Radiation therapy	9
Site of indicator lesions	
Pelvis	20
Abdomen	8
Liver	4
Chest	4
Skin	2
Extravisceral node	2
Bone	1
Measurement of indicator lesion	
Physical examination	16
Computed tomography	15
X-ray	4
Ultrasound	4
Nuclear scan	2

[1]Received Mar 25, 1985; revised June 24, 1985; accepted July 8, 1985.

[2]Supported in part by Public Health Service grants CA-22411, CA-13612, CA-04920, CA-12644, CA-13238, CA-03096. CA-20319, CA-21116, CA-28862, CA-22433, CA-12014, and CA-32102 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Mountain Cancer Treatment Center, Maryville, TN.

[4]University of Arizona Health Sciences Center, Tucson.

[5]Ohio State University Hospital, Columbus.

[6]University of Arkansas for Medical Sciences, Little Rock.

[*] *Reprint requests to:* Southwest Oncology Group (SWOG-8117), Operations Office, 5430 Fredericksburg Rd, Suite 618, San Antonio, TX 78229.

One hundred and eleven courses of therapy were given. The major toxicity was limited to leukopenia, with the median wbc count nadir being 2000 cells/μl (range, 800–6200). There were no life-threatening infections identified. Thrombocytopenia was not a major toxic effect, with only four (3.6%) courses associated with a platelet count < 100,000/μl. Nonhematologic toxicity was mild. Drug treatment-associated phlebitis (edema and pain) occurred only in patients receiving bisantrene by peripheral vein and occurred in all of these patients. There was no anaphylactic, cardiac, renal, or hepatic toxicity reported, with five patients receiving > 700 mg/m^2 and two patients receiving > 3000 mg/m^2. There were no drug-related deaths. There was one complete response lasting 30+ weeks. This response occurred in a patient previously treated with seven agents, including doxorubicin. Five patients had stable disease, with a median duration of 13 weeks (range, 9–16).

DISCUSSION

The clinical activity of bisantrene was minimal in these heavily pretreated patients. One complete response occurred in a patient previously treated with doxorubicin, which suggests a possible lack of cross-resistance between bisantrene and doxorubicin. Results of the human tumor cloning assay suggest that there may be a lack of cross-resistance between doxorubicin and bisantrene in ovarian cancer (2). Further study of bisantrene in ovarian cancer should be considered in a less heavily pretreated group to determine its activity and degree of cross-resistance with doxorubicin.

REFERENCES

1. CITARELLA RV, WALLACE RE, MURDOCK KC, ET AL. Antitumor activity of Cl 216,942: 9-10-anthracenedicarboxaldehyde bis (4,5-dihydro-1*H*-imidazole-2-yl)-hydrazone) dihydrochloride. *In* Abstracts of the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy, Abstract 23, 1980.
2. COWAN JD, VON HOFF DD, and CLARK GM. Activity of mitoxantrone and bisantrene in a human tumor cloning system. *In* New Anticancer Drugs: Mitoxantrone and Bisantrene (Rozencweig M, et al, eds). New York, Raven Press, 1983, pp 39–46.
3. VON HOFF DD, MYERS JW, KUHN J, ET AL. Phase I clinical investigation of 9,10-anthracenedicarboxaldehyde bis [(4,5-dihydro-1*H*-imidazol-2-yl) hydrazone]dihydrochloride (CL 216,942). Cancer Res 41:3118–3121, 1981.

Phase II Trial of Fludarabine in Patients With Epithelial Ovarian Cancer[1,2,3]

John J. Kavanagh,* C. Allen Stringer, Larry J. Copeland, David M. Gershenson, and Patton Saul[4]

Fludarabine phosphate (2-fluoro-ara-AMP) is the fluoridated derivative of adenine arabinosyl monophosphate. The fluoridation increases the drug's resistance to deamination, while the phosphate improves its solubility. The compound has in vitro activity against L1210 leukemia, P388 leukemia, and human LX-1 lung xenografts (1).

In phase I trials, the major side effect of fludarabine was myelosuppression, particularly neutropenia. The drug was well tolerated subjectively. An acceptable dosage was considered to be 20 mg/m^2 given every 12 hours times six doses (2). Myelosuppression was more severe in patients who had a creatinine clearance of < 75 ml/min. This is thought to be a result of decreased renal clearance of the drug (3).

MATERIALS AND METHODS

Twenty-nine patients who had refractory epithelial ovarian cancer received fludarabine. The drug was given in 125 ml of 5% dextrose in water over 30 minutes. One course consisted of six injections given 12 hours apart. The starting dose for patients who had received prior melphalan or radiation therapy or for those whose creatinine clearances were > 50 or < 70 ml/min was 15 mg/m^2 per injection (90 mg/m^2 per course). All other patients received 20 mg/m^2 per injection (120 mg/m^2 per course). Patients who had a creatinine clearance of < 50 ml/min were ineligible for the study.

The treatment course was repeated every 21 days, depending upon the patient's hematologic recovery. Dosage escalation was aimed at producing a nadir granulocytopenia of 1000 cells/mm^3 or a platelet count of 50,000-100,000/mm^3. Patient characteristics are listed in table 1.

TABLE 1.—Patient characteristics

Characteristic	No. of patients	%
Total evaluable	27	100
Median age in yrs (range): 51 (24-75)		
Performance status (Zubrod)		
0	4	15
1	13	48
2	8	30
3	2	7
Prior chemotherapy (cisplatin combinations)	13	87
Median No. of regimens: 2		
Median granulocyte count nadir (range): 984/mm^3 (174—4980)		
Courses with nadir of—		
> 500 to < 1000		35
≤ 500		14
Median platelet count nadir (range): 163,000/mm^3 (19,000—650,000)		
Courses with nadir of—		
> 50,000 to ≤ 100,000		15
≤ 50,000		4

RESULTS

Two of the 29 patients were inevaluable: one refused to return for evaluation and one died (from progressive malignancy) within a week after receiving the drug. There were no responses among the remaining 27 patients. One patient showed subjective improvement and modest lessening of ascites as measured by computerized tomography.

A total of 58 courses of fludarabine were given, with a median of two per patient. Dosage escalation was common during the trial. Of 14 patients started at a dose of 15 mg/m^2, seven (50%) had the dose escalated to 20 mg/m^2. Of 13 patients started at the higher dose, three (23%) had that dose increased to 25 mg/m^2. No patient received a dose > 25 mg/m^2.

The drug was subjectively well-tolerated, with minimal nausea. One patient discontinued therapy due to malaise associated with prolonged granulocytopenia. No acute

[1]Received Apr 9, 1985; accepted June 12, 1985.

[2]Supported in part by contract N01-CM-27550 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Fludarabine was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4]Departments of Gynecology (*J. J. Kavanagh, C. A. Stringer, L. J. Copeland, D. M. Gershenson,* and *P. Saul*) and Chemotherapy Research (*J. J. Kavanagh*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

* *Reprint requests to:* John J. Kavanagh, MD, Division of Gynecologic Oncology, H. Lee Moffitt Cancer Center and Research Institute, University of South Florida (Box 18), 12901 N 30th St, Tampa, FL 33612.

neurologic toxicity was observed. Neutropenia was clearly the dose-limiting side effect. Five patients (19%) were hospitalized with neutropenia and fever. One patient received seven courses of the drug and at the time of this writing had prolonged granulocytopenia of 10 weeks' duration. No therapeutic responses were seen.

Thus, we conclude that fludarabine at this dosage schedule is inactive against refractory epithelial ovarian cancer. Although subjectively well-tolerated, the compound causes significant neutropenia.

REFERENCES

1. HUTTON JJ, VON HOFF DD, KUHN J, ET AL. Phase I clinical investigation of 9-β-D-arabinofuranosyl-2-fluoroadenine 5'-monophosphate (NSC 312887), a new purine antimetabolite. Cancer Res 44:4183-4186, 1984.
2. KAVANAGH J, KRAKOFF I, and BODEY G. Phase I study of fludarabine (2-fluoro-ara-AMP). Eur J Cancer 21:1009-1011, 1985.
3. Minutes of the Phase I Working Group Meeting and Biochemical Modulators Advisory Group Meeting, Sept 19-22, 1983. Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, pp 141-158.

Phase II Trial of Diaziquone in Anthracycline-Resistant Adult Soft Tissue and Bone Sarcoma Patients: A Southeastern Cancer Study Group Trial[1,2]

Clara Chan,[3,4] Alfred Bartolucci,[5,*] Dean Brenner,[6] Cary Presant,[3,7] Enrique Davila,[8] John Carpenter,[5] F. Anthony Greco,[6] Gerald Clamon,[9] and Joseph Moore[10]

Diaziquone (AZQ) is a synthesized benzoquinone designed to have good lipid solubility, low ionization (to penetrate the CNS), and good water solubility (1,2). The exact mechanism of action is unknown, although it is thought to possess alkylating activity (3). The quinone structure suggests that free radical activity may account for some of its antitumor activity (4). Because of its presumed anthracycline-like activity, this compound was selected for phase II study in patients with soft tissue sarcoma by the Southeastern Cancer Study Group.

Forty-eight patients (table 1) with histologically proven soft tissue or bone sarcomas were treated with AZQ (20 mg/m^2 iv) on Days 1 and 8 of a 28-day cycle. Patients with poor marrow reserve, defined by prior irradiation > 3000 R or prior chemotherapy with more than two courses of a nitrosourea or mitomycin, received 15 mg/m^2 on Days 1 and 8. Doses were reduced for myelotoxicity and for renal or hepatic function impairment. All patients had measurable lesions by physical examination, chest x-ray, or computerized tomography prior to treatment. Standard response criteria were employed.

Forty patients with soft tissue sarcoma and five with bone sarcoma were evaluable for response and toxicity. No patients responded to this treatment. Thirteen patients had stable disease after two courses of treatment; the rest of the patients had disease progression. Myelotoxicity was the major, dose-limiting toxic effect (table 1), with one episode of severe, life-threatening myelosuppression in a patient who had been heavily pretreated. Nausea and vomiting were mild to moderate and easily controlled by antiemetic drugs. No other toxic effects were observed.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	48
Evaluable	45
Median age in yrs (range): 49 (22-69)	
Males/females	26/19
Median Karnofsky performance score (range): 80 (50-100)	
Prior treatment*	
Chemotherapy (single drug)	10
Chemotherapy (multiple drugs)	32
Radiation therapy	25
Mediastinal	4
Abdominal	3
Pelvic	5
Extremity	4
Other	9
Histologies	
Malignant fibrous histiocytoma	8
Leiomyosarcoma	13
Rhabdomyosarcoma	3
Liposarcoma	4
Synovial sarcoma	3
Osteogenic sarcoma	1
Other	13
Extent of metastatic disease*	
Lung	15
Liver	7
Gastrointestinal	9
Brain	1
Bone	8
Lymph nodes	7
Other sites	14
Toxicity*	
Wbc count nadir < 3000/mm^3	11
Wbc count nadir < 1500/mm^3	9
Platelet count nadir < 100,000/mm^3	5
Platelet count nadir < 50,000/mm^3	5
Nausea, vomiting	18

* One patient could fit into multiple categories.

[1]Received Apr 16, 1985; accepted June 12, 1985.

[2]Supported in part by Public Health Service grants CA-19657, CA-24456, CA-03013, CA-03177, CA-05641, CA-23909, CA-13237, CA-15584, CA-17027, CA-28169, CA-28138, CA-28171, CA-15578, CA-03376, CA-37577, CA-30247, CA-37546, and CA-20255 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]City of Hope Medical Center, Duarte, CA.

[4]*Present address:* Veterans Administration Medical Center, Washington, DC.

[5]Department of Biostatistics and Biomathematics (*A. Bartolucci*) and Comprehensive Cancer Center, Division of Hematology/Oncology (*J. Carpenter*), University of Alabama, Birmingham.

[6]Division of Oncology, Department of Medicine, Vanderbilt University, Nashville, TN.

[7]*Present address:* Wilshire Oncology Group, Los Angeles, CA, and the Central Los Angeles Community Clinical Oncology Program.

[8]Department of Oncology, School of Medicine, University of Miami, FL.

[9]University of Iowa Hospitals, Iowa City.

[10]Duke University School of Medicine, Durham, NC.

[*]*Reprint requests to:* Alfred Bartolucci, PhD, LBW Tumor Institute, Rm G-01, University of Alabama, Birmingham, AL 35294.

Despite the thought that quinone structure may result in response rates similar to those obtained with anthracyclines, AZQ was not active in a group of previously treated patients with soft tissue sarcoma. We attempted to have a representative sample of major sarcoma tissue subtypes to determine whether an individual subtype would respond to treatment. Unfortunately, AZQ has a < 20% response rate with a 5% rejection error. From these data we conclude that AZQ is inactive in common sarcomas and that further study with this compound will probably not prove fruitful.

REFERENCES

1. Clinical Brochure. Aziridinylbenzoquinone (NSC-182986). Bethesda, MD, National Cancer Institute, Aug 1979.
2. CHOU F, KHAN AH, and DRISCOLL JS. Potential central nervous system antitumor agents. Aziridinylbenzoquinones II. J Med Chem 19:1302-1308, 1976.
3. AKHTAR MH, BEGLEITER A, JOHNSON D, ET AL. Studies related to antitumor antibiotics. Part VI. Correlation of covalent cross-linking of DNA by bifunctional aziridinylbenzoquinones with their antineoplastic activities. Can J Chem 53:2891-2905, 1975.
4. SVINGE BA, APPEL PL, and POWIS G. Radical formation during cellular and subcellular metabolism of the chemotherapeutic agent AZQ. The Pharmacologist 22:177, 1980.

Phase II Trial of Diaziquone in Malignant Mesothelioma[1]

Robert T. Eagan,* Stephen Frytak, Ronald L. Richardson, Edward T. Creagan, and William C. Nichols[2]

Malignant mesothelioma is a disease which is both increasing in incidence (1) and difficult to treat (2). Data on responsiveness to chemotherapy are limited (2). We conducted a phase II evaluation of diaziquone (AZQ) based on a 1981 report (3) that the drug prolonged survival in the hamster mesothelioma 10-24 model to a degree comparable to azacitidine, a drug with some reported activity against human mesothelioma.

MATERIALS, METHODS, AND RESULTS

Twenty-one patients with unresectable and metastatic measurable or evaluable mesothelioma were entered in the study after obtaining written informed consent. One patient was subsequently declared ineligible when pancreatic carcinoma was found to be the correct diagnosis at autopsy. Of the 20 eligible patients, 18 had pleural and two had peritoneal mesothelioma. The characteristics of these 20 patients are shown in table 1. Prior to treatment with AZQ, patients had to have a wbc count $\geq 3500/mm^3$ and a platelet count $\geq 130,000/mm^3$, creatinine ≤ 2.0 mg/dl, normal direct bilirubin, no prior chemotherapy within 4 weeks, and an Eastern Cooperative Oncology Group (ECOG) performance score of 0-3. Patients with no prior chemotherapy began AZQ at a dose of 30 mg/m^2 iv every 4 weeks versus 25 mg/m^2 iv every 4 weeks for patients with prior chemotherapy. Dosages were scheduled for adjustment in an attempt to produce wbc count nadirs in the $2000-3000/mm^3$ range and/or platelet count nadirs in the $75,000-100,000/mm^3$ range. At present, 19 of 20 patients have progressed on AZQ and 14 of 20 are dead. No tumor regressions were noted. Treatment and survival results are shown in table 1. The mean number of treatments received was 1.9 (range, one to six). Nausea occurred in 45% of patients (mild in 35%), vomiting in 25% (mild in 20%), and stomatitis in 5%. The median wbc count nadir after Cycle 1 ($\times 10^3$ cells/μl) was 4.9 (range, 0.6-11.2; 41% $\leq$ 3.5) and after all cycles was 3.3 (range, 0.6-11.2; 53% $\leq$ 3.5). The median platelet count nadir after Cycle 1 ($\times 10^3$ cells/μl) was 193 (range, 57-658; 24% $\leq$ 130) and after all cycles was 143 (range, 22-570; 41% $\leq$ 130).

TABLE 1.—Pretreatment characteristics and data on response to treatment

Characteristic	No. of patients
Total	20
Prior chemotherapy	4
Median age in yrs (range): 59.5 (50-66)	
ECOG performance score	
0-1	7
2-3	13
Sex	
Male	16
Female	4
Site	
Pleural	18
Peritoneal	2
Best response	
Stable disease	12
Progressive disease	8
Median time to disease progression in days (range): 53 (13-305+)	
Median survival in days (range): 178 (13-305+)	

DISCUSSION

AZQ produced no tumor regressions among 20 patients with malignant mesotheliomas (95% confidence limits, 0%-17%). These data would suggest that the hamster mesothelioma 10-24 model may not be predictive for human mesothelioma and that AZQ, using the dosages and schedules employed, is not an active treatment for human mesothelioma.

REFERENCES

1. NEWHOUSE M. Epidemiology in asbestos-related tumors. Semin Oncol 8:250-257, 1981.
2. CHAHINIAN AP, PAJAK TF, HOLLAND JF, ET AL. Diffuse malignant mesothelioma. Prospective evaluation of 69 patients. Ann Intern Med 96:746-755, 1982.
3. HOLIAT SM, SMITH WE, HUBERT DD, ET AL. Chemotherapeutic trials with hamster mesothelioma 10-24: responses to azacitidine, aziridinylbenzoquinone, cisplatin, and PCNU. Cancer Treat Rep 65:1113-1115, 1981.

[1]Received Apr 17, 1985; revised July 1, 1985; accepted July 9, 1985.
[2]Division of Medical Oncology, Mayo Clinic, Rochester, MN.

**Reprint requests to:* Robert T. Eagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55905.

High-Dose Medroxyprogesterone Acetate in Patients With Renal Adenocarcinoma and Measurable Lung Metastases: A Phase II Study[1]

Mogens Kjaer[2,*] and Peter L. Frederiksen[3,4]

The role of medroxyprogesterone acetate (MPA) in the treatment of metastatic renal adenocarcinoma remains controversial. In the early 1970s, Bloom (1) reported response rates of up to 27% with oral doses of 300 mg daily. Since then, these data have not been reproduced. The concept of high-dose MPA, 500–1000 mg daily im, was introduced in 1976 by Pannuti et al (2) in the treatment of breast cancer; these high doses seem to be beneficial compared to conventional doses. Two earlier studies of high-dose MPA in renal adenocarcinoma have shown very limited activity, but the measurability of the lesions was not clarified (3,4).

PATIENTS AND METHODS

Twenty-one patients with histologically proven renal adenocarcinoma and clearly measurable lung metastases on plain chest x-ray and/or computerized tomographic scan who had not been treated earlier with chemotherapy or hormone therapy were included in the study after giving informed consent. Patient characteristics, treatment schedule, and overall results are shown in table 1. The following examinations were performed before the start of therapy: physical examination, hematology, liver biochemistry, creatinine, chest x-ray, computerized tomographic scan if indicated, bone scan, and ultrasound scan of the kidney bed in nephrectomized patients.

Patients were evaluated every 4 weeks during therapy, and response was classified according to World Health Organization criteria. Plasma levels of MPA were determined monthly (5).

TABLE 1.—Patient characteristics, treatment schedule, and response*

Characteristic	Male	Female
Total	11	10
Median age in yrs (range)	56 (36–73)	61 (49–66)
Earlier nephrectomy	6	7
Median time from nephrectomy to MPA (range)	43 mos (0 days–7 yrs)	10 mos (0 days–3 yrs)
Median performance status at treatment start (range)	1 (0–3)	1 (0–2)
Metastatic disease outside lungs	6	6
Median time with known metastatic disease before MPA (range)	150 days (0 days–3 yrs)	60 days (0 days–10 mos)
Median duration of treatment in days (range)†	90 (48–320)	115 (28–259)
Response	0	1‡

*Unless otherwise specified, values = No. of patients.

†Treatment schedule for MPA induction was 500 mg/day im for 28 days; schedule for maintenance of treatment was 1000 mg/wk im.

‡Partial response.

[1]Received May 7, 1985; revised July 9, 1985; accepted July 23, 1985.

[2]Department of Oncology II, Radiumstationen, Finsen Institute, Copenhagen, Denmark.

[3]Department of Oncology and Radiotherapy, Copenhagen County Hospital, Herlev, Denmark.

[4]We thank Farmitalia Carlo Erba, Copenhagen, for the supply of medroxyprogesterone acetate (Farlutal), and Dr. Inge Hesselius, Uppsala, Sweden, for measurements of MPA plasma concentrations.

*Reprint requests to: Mogens Kjaer, MD, Department of Oncology II, Radiumstationen, Finsen Institute, 49 Strandboulevarden, DK-2100 Copenhagen Ø, Denmark.

RESULTS

One patient, a 66-year-old woman, achieved a partial response (after 109 days of treatment) lasting 135+ days. She died after 244 days with pulmonary partial response but hepatic progressive disease. Eleven patients went off study with pulmonary progressive disease after a median duration of treatment of 90 days (range, 28–320). Seven patients went off study with no change in the lungs but progressive disease outside of the lungs and general deterioration after a median duration of treatment of 62 days (range, 38–193). Two patients stopped treatment with no change after 180 and 259 days, respectively, and are followed without therapy.

Side effects were minimal (usually a weight gain of 2–3 kg). No analgesic effect or positive effects on the general condition of the patients receiving MPA were recorded.

MPA reached steady levels in plasma after 1 month of therapy in all patients, but with a considerable individual variation in the plasma concentrations. The median plasma concentration after 2 months was 100 nmols/L (range, 42–324).

CONCLUSION

We conclude from the present study that high-dose MPA given to patients with advanced renal adenocarcinoma and clearly measurable lung metastases has only minimal activity (5% response rate; 95% confidence limits, 0.12%–23.82%). In our opinion, MPA should not be used in the treatment of renal adenocarcinoma, neither in the metastatic nor the adjuvant setting. MPA is expensive and it does not work except for an occasional response. To achieve a general positive effect on the well-being and appetite of the patients, we recommend prednisone, 30–40 mg/day orally.

REFERENCES

1. BLOOM HJG. Medroxyprogesterone acetate (Provera) in the treatment of metastatic renal cancer. Br J Cancer 25:250–265, 1971.
2. PANNUTI F, MARTONI A, POLLUTI E, ET AL. Massive dose progestational therapy in oncology (medroxyprogesterone). Preliminary results. Panminerva Med 18:129, 1976.
3. PANNUTI F, MARTONI A, and CRICCA A. Treatment of renal clear cell carcinoma by high doses of medroxyprogesterone acetate (MPA): pilot study. IRCS Med Sci 6:177, 1978.
4. WICKLUND H. High-dose medroxyprogesterone acetate in patients with advanced renal cell carcinoma. *In* Proceedings of the International Symposium on MPA, Geneva, Switzerland, Feb 1982. Excerpta Medica International Congress Series No. 611, pp 420–424.
5. HESSELIUS I, and JOHANSSON EDB. Medroxyprogesterone acetate (MPA) plasma levels after oral and intramuscular administration in a long-term study. Acta Obstet Gynecol Scand (suppl)101:65–70, 1981.

Cardiotoxicity Associated With 4′-Deoxydoxorubicin

Cardiac toxicity is a life-threatening, cumulative, and dose-limiting toxic effect of doxorubicin. 4′-Deoxydoxorubicin (DxDx) is a substituted analog of doxorubicin (1) and is undergoing phase II clinical testing in the United States. DxDx is less cardiotoxic in animals than the parent compound. In our phase II trial of DxDx using patients with pancreatic carcinoma previously unexposed to irradiation or chemotherapy, one case of subclinical cardiotoxicity has occurred, which was manifested as a sustained decrement in the cardiac ejection fraction (Ejfx) determined by gated radionuclide angiography and was substantiated by two-dimensional echocardiography.

The patient was a 54-year-old woman without previous history of cardiac disease who had progressive, painless jaundice. At exploratory laparotomy, unresectable carcinoma of the pancreatic head was found, and she underwent gastrojejunostomy, choledochojejunostomy, and cholecystectomy without postoperative complication. The patient's ECG prior to chemotherapy was normal, and her resting Ejfx was 64%. She gave informed consent and entered a phase II trial of DxDx at a starting dose of 35 mg/m^2. Cumulative DxDx dose was 184 mg/m^2. Three weeks after her last dose, Ejfx was 54% with diffuse wall motion abnormalities and she had progressive cancer and was taken off the study. One month later, her Ejfx was 51%, serum electrolytes were normal, ECG was unchanged, and M-mode and two-dimensional echocardiography did not show valvular or pericardial disease and confirmed the diffuse wall motion abnormalities. Physical examination of the heart was normal; endomyocardial biopsy was not clinically warranted.

Although other etiologies for global myocardial dysfunction may exist in this patient, cardiac damage produced by DxDx is the most likely explanation. This observation is consistent with prior studies which have shown that loss of Ejfx is an early sign of anthracycline-induced myocardial dysfunction (2). Results from our ongoing trials and other trials which employ routine endomyocardial biopsy will be necessary to establish both the efficacy and toxicity of this doxorubicin analog and the specificity of the endomyocardial biopsy for DxDx-induced cardiac toxicity.

REFERENCES

1. Investigator's Brochure. 4′-Deoxy-doxorubicin. Bethesda, MD, National Cancer Institute, 1983.
2. ALEXANDER J, DAINAK N, BERGER HJ, ET AL. Serial assessment of doxorubicin cardiotoxicity with quantitative radionuclide angiocardiography. N Engl J Med 300:278-283, 1979.

Douglas Blayney[1,2,*]

July 15, 1985

[1]Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte, CA.
[2]Recipient of a Career Development Award from the American Cancer Society.
[*]*Reprint requests to:* Douglas Blayney, MD, Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, 1500 E Duarte Rd, Duarte, CA 91010.

Response of Glucagonoma Syndrome to Lomustine

We have previously reported a patient with glucagonoma syndrome who had unusual neurological symptoms and signs indicative of a diffuse encephalopathy. At the time of initial presentation, progressive weakness, lethargy, dull wit, slow speech, and inability to concentrate were noted. He also noted some bladder and bowel incontinence on some occasions. He had an unsteady, stiff gait. At that time, his liver span was 13 cm in the midclavicle line and he had plaque-like erythematous lesions on the thighs, heels, calves, arms, chest, and buttocks. On neurological examination he had mild dementia and impaired immediate recall. There was evidence of mild optic atrophy; poorly sustained nystagmus to lateral gaze bilaterally; positive snout reflex; mild ataxia of the limbs, evident chiefly in the arms on finger-to-nose testing; truncal ataxia; and hyperactive deep-tendon reflexes in all limbs, with spastic weakness of the legs and bilateral Babinski signs (1). As reported previously, the technetium-99m sulfur colloid liver scan showed multiple filling defects. The patient was treated with 5-FU and streptozocin. The patient had a complete clinical, radiological, biochemical, and neurological remission.

This patient remained asymptomatic and in complete remission for about 4 years. His relapse at that time was manifested by recurrence of dermatitis, stomatitis, deterioration of his diabetes mellitus, and recrudescence of

his neurological symptoms. The major abnormalities in the neurological examination at that time included slow speech, decreased ability to concentrate, truncal ataxia, and spastic weakness of the legs with positive bilateral Babinski signs. In October 1982, the plasma immunoreactive glucagon (IRG) level was 4 ng/ml (normal glucagon level, 0.1 ± 0.04 ng/ml).

The patient was treated with 5-FU plus streptozocin for one cycle and exhibited no improvement. Similarly, dacarbazine was also unsuccessful in inducing any remission. Therefore, on November 12, 1982, the patient was begun on lomustine at 70 mg/m^2 orally, every 3 weeks. Within 2 months the patient had a remarkable clinical, neurological, and biochemical response. The hepatomegaly and filling defects on liver/spleen scan disappeared and neurologically the leg stiffness and truncal ataxia improved. The plasma IRG level decreased to 0.15 ng/ml. He has continued to do well on this regimen, although the nitrosourea dosages and frequency of administration have been reduced because of cumulative bone marrow toxicity.

Recently, Graner and Abraira (2) reported that glucagon, like insulin, possibly is actively transported from the blood into the CSF. In our patient, CSF glucagon level was 215 pg/ml, with simultaneous plasma glucagon of 1716 pg/ml, ie, a ratio of 1:8. Graner and Abraira reported that in a normal individual the CSF glucagon was 53 pg/ml, with CSF glucagon level averaging 28% of normal serum levels. More recently, glucagon receptors have been identified in the rat brain (3). Thus, in our patient the CSF glucagon level was higher than those reported by Graner and Abraira in normal individuals. It is therefore possible that the neurological syndrome seen in our patient may be related to the higher CSF glucagon level. Our observation that lomustine caused an improvement in neurological syndrome concomitant with a decline in plasma IRG supports this observation.

A review of the literature did not reveal any reports on the efficacy of lomustine in islet cell tumors. Our personal observations on two additional patients with islet cell tumors suggest that lomustine may play a role in the management of islet cell tumors. Lomustine thus merits further trials in islet cell tumors.

REFERENCES

1. KHANDEKAR JD, OYER D, MILLER HJ, ET AL. Neurologic involvement in glucagonoma syndrome. Cancer 44:2014-2016, 1979.
2. GRANER JL, and ABRAIRA C. Glucagon in the cerebrospinal fluid. N Engl J Med 312:994-995, 1985.
3. HOOSEIN NM, and GURD RS. Identification of glucagon receptors in rat brain. Proc Natl Acad Sci USA 81:4368-4372, 1984.

Janardan D. Khandekar[1,*]
P. Sriratana[2]

July 19, 1985

[1]Division of Medical Oncology, Evanston Hospital, IL, and Department of Medicine, Northwestern University Medical School, Chicago, IL.
[2]Bloomington, IL.
Reprint requests to: Janardan D. Khandekar, MD, Evanston Hospital, 2650 Ridge Ave, Evanston, IL 60201.

ANNOUNCEMENTS

Gastrointestinal Oncology—1986: An Advanced Postgraduate Course

Memorial Sloan-Kettering Cancer Center, New York, New York, April 3–4, 1986

The Continuing Medical Education Office at Memorial Sloan-Kettering Cancer Center is offering a course on gastrointestinal oncology. Directors of the course are Sidney J. Winawer, MD, and Robert C. Kurtz, MD. This program is aimed at physicians and surgeons who manage patients with gastrointestinal malignancies and gastrointestinal complications of other diseases including AIDS.

For further information, contact: CME Conference Planning Office, C-180, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telephone: 212-794-6754).

1986 Annual Meeting of the American Radium Society

San Francisco, California, April 6–10, 1986

For further information, contact: Ms. Susanne Bohn, Executive Secretary, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

American Cancer Society National Conference on Urologic Cancer—1986

Philadelphia, Pennsylvania, April 9–11, 1986

The American Cancer Society is sponsoring this conference on urologic cancer, to be held at the Adam's Mark Hotel in Philadelphia, PA. Topics included in the conference are: the role of staging in urologic cancer, integrated management of localized cancers, strategies for the management of recurrent and advanced cancers, and future developments and new research in genitourinary cancers.

The American Cancer Society is accredited by the Accreditation Council for Continuing Medical Education. This continuing medical education activity meets the criteria for 15 hours in Category 1 of the Physician's Recognition Award of the American Medical Association. Continuing education credits have been requested from the American Academy of Family Physicians and the American Osteopathic Association.

The meeting will be open to primary care physicians, specialists in oncology, nurses, and students. Other interested health care professionals are welcome. Advance registration is requested. There will be an advance registration fee of $200.00 for physicians and dentists and $100.00 for all other health professionals. For registration received after April 1, 1986, the fee will be $250.00 for physicians and dentists and $150.00 for all other health professionals. A processing fee of $50.00 will be charged for cancellations after initial registration. Checks or money orders only will be accepted. There is no registration fee for students, interns, residents, and American Cancer Society Fellows or Career Development Awardees.

For further information, contact: The American Cancer Society, National Conference on Urologic Cancer—1986, 90 Park Ave, New York, NY 10016.

Lung Cancer Update Conference

Baltimore, Maryland, April 11–12, 1986

The Division of Thoracic and Cardiovascular Surgery of the University of Maryland School of Medicine and the American Cancer Society, Maryland Division, Inc, are sponsoring this 2-day continuing education conference on lung cancer. This course presents the progress made in the diagnosis and management of lung cancer over the past 25 years, as well as an update on the current methods of classifi-

cation, therapeutic modalities, and surgical results. The course also offers an outlook into the future advances of lung cancer.

For further information, contact: Program of Continuing Education, University of Maryland School of Medicine, 10 S Pine St, Suite 300, Baltimore, MD 21201 (telephone: 301-528-3956).

International Meeting on Cancer Metastasis

Vitoria, Spain, April 14-17, 1986

The University of the Basque Country in northern Spain is organizing an international meeting on the therapeutic aspects of cancer metastasis in Vitoria April 14-17. Topics for discussion include: immunological and therapeutic aspects in cancer metastasis; host factors in metastasis; organ specificity; genetic heterogeneity of tumor cells; phenotypic heterogeneity of tumor cells; cell membranes; and tumor cell-connective tissue interactions.

For further information, contact: Meeting Secretariat, Apartado 1299, 48080 Bilbao, Spain.

First International Congress on Cancer and Hormones

Rome, Italy, April 23-27, 1986

This conference will include such topics as newer diagnostic and therapeutic approaches; epidemiology of endocrine-related cancer; hormone receptors and tumors; oncogenes, growth factors, and small effectors; hormones and cell proliferation; and cancers arising in endocrine glands.

For further information, contact: Stefano Iacobelli, MD, Laboratorio di Endocrinologia Molecolare, Istituto di Clinica Ostetrica e Ginecologica, Università Cattólica del S. Cuore, L.go Agostino Gemelli, 8, 00168 Rome, Italy [telephone: (06) 33.054.496; telex: 611330 UNCATRO].

EORTC Symposium on GI Tract Cancer—Update on Combined Modality Therapy

Heidelberg, Federal Republic of Germany, May 29-30, 1986

The European Organization for Research on Treatment of Cancer Gastrointestinal Tract Cancer Cooperative Group is sponsoring this symposium which will focus on the role of perioperative and adjuvant therapeutic strategies in the treatment of cancer of the gastrointestinal tract. The experimental basis of multidisciplinary treatment will be explored and correlated with its latest application in various clinical settings involving primary, recurrent, or disseminated disease. Results of recent EORTC trials and state-of-the-art lectures will be presented. The topics of the meeting will be discussed in free papers and in poster sessions.

Seating is limited and preregistration is recommended. The registration fee is $120.00 (before March 1, 1986) and will be $150.00 thereafter.

For further information, contact: Dr. P. Schlag, Chirurgische Klinik, Universität Heidelberg, Im Neuenheimer Feld 110, 6900 Heidelberg, Federal Republic of Germany (telephone: 6221-56.21.11).

Second International Symposium on Prostatic Cancer

Paris, France, June 16-18, 1986

The Second International Symposium on Prostatic Cancer will be held at the Intercontinental Hotel, 3, rue de Castiglione, Paris, France. Scientific contributions, poster presentations, and films will be accepted. Category 1 and AUA Continuing Medical Education credits will be awarded.

For further information, contact: Saad Khoury, MD, Clinique Urologique, Hôpital de la Pitié, 83, Boulevard de l'Hôpital, 75634 Paris Cedex 13, France (telephone: 88-1-570.30.78); or James P. Karr, PhD, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716-845-2317).

Sixth Annual Symposium on Fine Needle Aspiration

Budapest, Hungary, August 9-16, 1986

The Department of Pathology at the University of California School of Medicine in San Francisco and the Society of Hungarian Pathologists in cooperation with the California Society of Pathologists are sponsoring the Sixth Annual Symposium on Fine Needle Aspiration, to be held at the Atrium Hyatt

Hotel, Budapest, Hungary. This symposium is designed to provide a comprehensive introduction to fine needle aspiration biopsy techniques and interpretive criteria. Tuition includes lectures, extensive syllabus, Kodachrome study set, FNA performance, microscopes, microscopy sessions, and case presentations. The symposium offers ACCME/AMA/CMA Category 1 credits.

For further information, contact: Extended Programs in Medical Education, Rm 569-U, University of California School of Medicine, San Francisco, CA 94143 (telephone: 415-666-4251).

Bone and Cancer: International Congress in Hanasaari

Helsinki, Finland, October 9-11, 1986

For further information, contact: European Forum for Orthopaedic Science Congress Bureau, PO Box 4, SF-00661, Helsinki, Finland.

Fifth NCI-EORTC Symposium on New Drugs in Cancer Therapy

Amsterdam, The Netherlands, October 22-24, 1986

This symposium will be organized by the European Organization for Research on Treatment of Cancer (EORTC) and by the National Cancer Institute (NCI), National Institutes of Health. The following topics will be discussed: screening and pharmacology, formulation, phase I trials and clinical pharmacology, phase II trials of new cytostatic agents, new analogs, and biological response modifiers.

For further information, contact: Symposium Secretariat, EORTC-New Drug Development Office, Department of Oncology, Free University Hospital, De Boelelaan 1117, NL-1081 HV Amsterdam, The Netherlands [telephone: (20) 548-5193, 548-5192].

Chemotherapy Foundation Symposium VII—Innovative Cancer Chemotherapy for Tomorrow

New York, New York, November 12-14, 1986

The Sheraton Centre Hotel in New York City will be the location for the Chemotherapy Foundation Symposium VII—Innovative Cancer Chemotherapy for Tomorrow, to be held November 12-14, 1986. Individual sessions will be devoted to postmenopausal breast cancer polychemotherapy, differentiation chemotherapy, chemoimmunotherapy, and new phase II drugs.

The 3-day symposium is being sponsored by the Division of Medical Oncology, Department of Medicine, Department of Neoplastic Diseases, and the Page and William Black Post-Graduate School of Medicine of the Mount Sinai School of Medicine (CUNY).

For further information, contact: Director, The Page and William Black Post-Graduate School of Medicine, One Gustave L. Levy Place, New York, NY 10029 (telephone: 212-650-6772).

Third International Conference on Malignant Lymphoma: Current Status and Prospects

Lugano, Switzerland, June 10-13, 1987

The Palazzo dei Congressi is the location for this series of lectures and workshops dealing with the diagnosis and treatment of Hodgkin's and non-Hodgkin's lymphoma in adults and children. Most lectures will be given by invited speakers. The program will include free presentations, workshops, and a poster session. The deadline for the receipt of abstracts is *January 15, 1987.*

For further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland (telephone: 091-23-62-10).

☆ U.S. GOVERNMENT PRINTING OFFICE: 1986-491-283:10018

To: CTR-CAN
SUPERINTENDENT OF DOCUMENTS
U.S. Government Printing Office
Washington, D.C. 20402

Subscription prices:

$29 per year domestic
$36.25 per year foreign

Make checks payable to the Superintendent of Documents

☐ I want to receive my own copy of the *Cancer Treatment Reports* for the next year.

☐ Here's my check for $_____ made out to the Superintendent of Documents.

Name _____

Address _____

City _____ State_____ Zip_____

Charge my credit card ☐ VISA ☐ MasterCard ☐ Deposit Account ☐ Choice

Credit Card No._____

Expiration Date _____

Signature _____

International Cancer
Research Data Bank
Branch

National Cancer Institute

ONCOLOGY OVERVIEWS

SELECTED ABSTRACTS ON CANCER RESEARCH TOPICS

WHAT ARE ONCOLOGY OVERVIEWS?

- Retrospective bibliographies containing 100–500 selected abstracts of recent cancer research publications on narrowly focused topics.

WHAT RESEARCH AREAS ARE COVERED?

- 15 topics per year, selected for high current interest to cancer researchers, 5 topics in each of three series:
  - — Cancer diagnosis and therapy
  - — Carcinogenesis
  - — Cancer virology/immunology/biology
- Selected topics are listed on the attached order form.

WHICH SOURCES ARE USED?

- Approximately 3,000 biomedical journals as well as books, theses, meetings abstracts, etc.
- Abstracts in ONCOLOGY OVERVIEWS are also available via on-line searching of the ICRDB computer database CANCERLIT, accessible at over 4,000 locations through the National Library of Medicine's computer system.

WHO PREPARED ONCOLOGY OVERVIEWS?

- Scientists and consultant researchers affiliated with two Cancer Information Dissemination and Analysis Centers (CIDACs).

WHAT CAN ONCOLOGY OVERVIEWS DO FOR ME?

- Rapidly update your knowledge of "hot" research topics.
- Provide an in-depth survey of published research in each topic area.

HOW CAN I RECEIVE ONCOLOGY OVERVIEWS?

- By registering an ongoing cancer research project on a form available from the ICRDB Program. Each ONCOLOGY OVERVIEW is automatically mailed to investigators working in the appropriate subject area.

  OR

- ONCOLOGY OVERVIEWS may be purchased directly from NTIS using the attached order form. Special rates are available for series subscriptions, which ensure automatic delivery of all ONCOLOGY OVERVIEWS within the series as new issues are published.

FOR MORE INFORMATION ON BACK TITLES WRITE TO:

ICRDB Branch
National Cancer Institute
Building 82, Room 103
International Cancer Information Center
Bethesda, Maryland 20892
Phone: (301) 496-7403

The following ONCOLOGY OVERVIEWS are available from the National Technical Information Service (NTIS). Prices for paper copies are listed with each title; microfiche prices are $4.50, but are $3.00 if followed by an asterisk (*). Double listed price for delivery outside the North American continent. For your convenience, mail your check or money order directly to NTIS. You may also use your American Express, MasterCard, Visa, or an NTIS Deposit Account.

MAIL TO:

National Technical Information Service
Subscription Department
U.S. DEPARTMENT OF COMMERCE
5285 Port Royal Road
Springfield, Va. 22161

PURCHASER:

Name _____

Organization _____

Address _____

City, State, Zip _____

Telephone (____) _____

For nonsubscription orders, please add $3 domestic ($4 foreign) for shipping and handling.

TOTAL ORDER $ _____

☐ Check enclosed for $ _____

☐ Charge my NTIS deposit account no. _____

☐ Send information on establishment of NTIS Deposit Accounts and Standing Orders _____

☐ Purchase order number _____

☐ Charge to my (circle one):

MasterCard Visa American Express

Credit Card Number _____ Expiration Date

_____

Signature for Credit Card

Cancer Diagnosis and Therapy Series

☐ PB86-922800	$3.50	Each Series. Available as a standing order‡
☐ PB84-922809	$8.00	Management of Pain in the Cancer Patient (OT-84/09)
☐ PB84-922808	$8.00	Postoperative Adjuvant Therapy of Breast Cancer (OT-84/08)
☐ PB84-922807	$11.00	Biological, Hormonal and Immunological Markers in Breast Cancer Patients (OT-84/07)
☐ PB84-922806	$11.00	Diagnosis and Treatment of Testicular Cancer (OT-84/06)
☐ PB84-922805	$6.50	Neuropsychiatric Assessment and Care of Cancer Patients (OT-84/05)
☐ PB84-922804	$8.00	Radiolabeled Antibodies in Cancer (OT-84/04)
☐ PB84-922803	$8.00	Adjuvant Chemotherapy of Osteosarcoma (OT-84/03)
☐ PB84-922802	$11.00	Diagnosis and Treatment of Ovarian Cancer (OT-84/02)
☐ PB84-922801	$8.00	Neutrons and Charged Particles in Radiotherapy (OT-84/01)
☐ PB83-922809	$8.00	Interstitial and Surface Brachytherapy (OT-83/09)
☐ PB83-922808	$6.50	Diagnosis and Treatment of Kaposi's Sarcoma (OT-83/08)
☐ PB83-922807	$8.00	Psychosocial Care of Pediatric and Adolescent Cancer Patients (OT-83/07)
☐ PB83-922806	$9.50	Diagnosis and Treatment of Wilms' Tumor (OT-83/06)
☐ PB83-922805	$9.50	Prognostic Factors, Chemotherapy and Chemoimmunotherapy of Malignant Melanoma (OT-83/05)
☐ PB83-922804	$6.50	Oncology Nursing (OT-83/04)
☐ PB83-922803	$10.00	Diagnosis and Treatment of Pediatric and Adolescent Gynecologic Malignancies (OT-83/03)
☐ PB83-922802	$13.00	Diagnosis and Treatment of Gastric Cancer (OT-83/02)
☐ PB83-922801	$10.00	Oligoleukemia and Other Preleukemic Conditions (OT-83/01)
☐ PB82-922813	$11.50	Interferon: Clinical Trials and Related Preclinical Research (OT-82/13)
☐ PB82-922812	$6.00*	Diagnosis and Treatment of Hepatocellular Carcinoma (OT-82/12)
☐ PB82-922811	$6.00*	Treatment of Bladder Cancer (OT-82/11)
☐ PB82-922810	$7.25*	Diagnosis and Treatment of Pancreatic Cancer (OT-82/10)
☐ PB82-922809	$4.50*	The Diagnosis and Treatment of Ocular Melanomas (OT-82/09)
☐ PB82-922808	$6.00*	The Role of Thymic Factors in Cancer (OT-82/08)
☐ PB82-922807	$5.25*	Diagnosis and Treatment of Renal Cancer (OT-82/07)
☐ PB82-922806	$6.00*	Diagnosis and Treatment of Pituitary Tumors (OT-82/06)
☐ PB82-922805	$5.25*	Diagnosis and Treatment of Malignant Pleural Effusions (OT-82/05)
☐ PB82-922804	$8.00*	Intra-Arterial Chemotherapy in Cancer Treatment (OT-82/04)
☐ PB82-922803	$4.50*	Diagnosis and Treatment of Bronchoalveolar Cancer of the Lung (OT-82/03)
☐ PB82-922802	$6.00*	Treatment of Prostatic Cancer (OT-82/02)
☐ PB82-922801	$6.50*	Supportive Care of the Cancer Patient: Management of Infectious and Hematologic Complications (OT-82/01)

Carcinogenesis Series

☐ PB86-922900	$3.50	Each Series. Available as a standing order‡
☐ PB84-922908	$11.00	Ultraviolet-Induced Skin Cancer (OK-84/08)
☐ PB84-922907	$14.00	The Enzymatic Activation and Detoxication of Polycyclic Aromatic Hydrocarbons (OK-84/07)
☐ PB84-922906	$12.50	Risk of Cancer from Exposure to Low-Level Ionizing Radiation (OK-84/06)
☐ PB84-922905	$11.00	Carcinogenic Risk from Air Pollution by Engine Exhaust and Fuel Evaporation (OK-84/05)
☐ PB84-922904	$8.00	Epidemiologic Studies of Racial Differences in Predisposition to Neoplasia: I. Blacks and Whites in the United States of America (OK-84/04)
☐ PB84-922903	$9.50	Prostaglandins and Carcinogenesis (OK-84/03)
☐ PB84-922902	$12.50	Transforming Genetic Sequences (OK-84/02)
☐ PB84-922901	$8.00	Enzymatic Methylation of DNA in Eukaryotes (OK-84/01)
☐ PB83-922904	$8.00	The Role of Bile Acids in the Promotion of Gastrointestinal Carcinogenesis (OK-83/04)
☐ PB83-922903	$11.00	Cellular Responses to Damaged DNA Other Than Excision Repair (OK-83/03)
☐ PB83-922902	$8.00	Methodology in Use for the Assessment of Carcinogenic Risk: II. Radiation (OK-83/02)
☐ PB83-922901	$11.50	Methodology in Use for the Assessment of Carcinogenic Risk: I. Chemical Agents (OK-83/01)
☐ PB82-922920	$10.00	Chromatin Alterations Associated with Carcinogenesis (OK-82/20)
☐ PB82-922919	$5.25*	Excision Repair of DNA Damage: III. The Regulation of Excision Repair (OK-82/19)
☐ PB82-922918	$6.00*	Excision Repair of DNA Damage: II. Human Genetic Defects in Excision Repair (OK-82/18)
☐ PB82-922917	$6.50*	The Role of Selected Dietary Factors in Carcinogenesis (OK-82/17)
☐ PB82-922916	$6.00*	Excision Repair of DNA Damage: I. Enzymology and Mechanisms of Excision Repair (OK-82/16)
☐ PB82-922915	$7.25*	Neurocarcinogenesis (OK-82/15)
☐ PB82-922914	$5.25*	Mutagens and Carcinogens in Cooked, Smoked, and Charred Foods (OK-82/14)
☐ PB82-922913	$7.25*	Phorbol Ester Action in Cell Culture (OK-82/13)
☐ PB82-922912	$6.00*	Naturally Occurring Dietary Carcinogens of Plant Origin (OK-82/12)

Cancer Virology/Immunology/Biology Series

☐ PB86-923000	$3.50	Each Series. Available as a standing order‡
☐ PB84-923005	$9.50	Intercellular Communication Related to Neoplasia (OB-84/05)
☐ PB84-923004	$9.50	Effects of Therapy for Primary Neoplasms on the Development of Second Malignant Neoplasms (OB-84/04)
☐ PB84-923003	$9.50	The Stem Cell Origin of Neoplasia (OB-84/03)
☐ PB84-923002	$12.50	Mechanisms of Metastasis (OB-84/02)
☐ PB84-923001	$11.00	Tumor Heterogeneity (OB-84/01)
☐ PB83-923007	$12.50	Immunomodulation in Cancer (OB-83/07)
☐ PB83-923006	$11.00	Epidermal Growth Factors (OB-83/06)
☐ PB83-923005	$8.00	Tumor Angiogenesis (OB-83/05)
☐ PB83-923004	$8.00	Rearrangements of DNA Sequences as They Occur in Nature: Potential Models for Differentiation and Tumorigenesis (OB-83/04)
☐ PB83-923003	$10.00	Protein Kinases Associated with Growth, Differentiation, and Transformation (OB-83/03)
☐ PB83-923002	$10.00	Non-Histone Proteins in Control and Malignant Cultured Cells (OB-83/02)
☐ PB83-923001	$10.00	Modulation of Cancer by Superoxide Dismutase (OB-83/01)
☐ PB82-923020	$16.00	DNA Viral Transforming Proteins (OB-82/20)
☐ PB82-923019	$11.50	Endogenous Oncornaviruses and Genes (OB-82/19)
☐ PB82-923018	$13.00	Membrane Glycoproteins in Control and Malignant Cells (OB-82/18)
☑ PB82-923017	$6.00*	The Viral Etiology of Cervical Cancer (OB-82/17)
☐ PB82-923016	$6.00*	Reverse Transcriptase (OB-82/16)
☐ PB82-923015	$6.00*	Tumor-Related Ferritins (OB-82/15)
☐ PB82-923014	$5.25*	Mitochondrial Respiration in Tumor Cells and Related Systems (OB-82/14)
☐ PB82-923013	$8.00*	Natural Killer Cells (OB-82/13)
☐ PB82-923012	$6.00*	Monoclonal Antibodies (OB-82/12)
☐ PB82-923011	$5.25*	The Role of Proteases in Metastasis (OB-82/11)
☐ PB82-923010	$6.50*	The Biochemistry of Cancer-Related Collagens (OB-82/10)
☐ PB82-923009	$6.50*	Interferon: Preclinical Studies of Anticellular, Antitumor and Immunomodulatory Activities (OB-82/09)
☐ PB82-923008	$6.00*	Mechanisms of Resistance to Methotrexate and Other Folate Antagonists (OB-82/08)
☐ PB82-923007	$6.50*	Liposomes (OB-82/07)
☐ PB82-923006	$6.50*	Interferon: Preclinical Studies of Antiviral Activity (OB-82/06)
☐ PB82-923005	$6.00*	Hepatitis B Virus (OB-82/05)
☐ PB82-923004	$6.00*	Endogenous Polypeptide Growth Factors (OB-82/04)

‡Foreign series orders are $5.25 each.

A Publication of the National Cancer Institute

NIH Publication No. 86-22